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DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

2026-4236US1

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/242202

INTERNATIONAL APPLICATION NO.  
PCT/US97/14306INTERNATIONAL FILING DATE  
14 August 1997 (14.08.97)PRIORITY DATE CLAIMED  
14 August 1996 (14.08.96)

## TITLE OF INVENTION

A VECTOR FOR POLYNUCLEOTIDE VACCINES

## APPLICANT(S) FOR DO/EO/US

Edward L. NELSON and Peter J. NELSON

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
- a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
- b. ☐ has been transmitted by the International Bureau.
- c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
- a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
- b. ☒ have been transmitted by the International Bureau.
- c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
- d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

## Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment. (with Exhibits A, B & C)
- ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:- Copy of the first page of the published International Application No. WO 98/06863;
- Copy of the International Preliminary Examination Report;
  - Disk containing Sequence Listing;
  - Statement Under 37 CFR §1.821(f) Or §1.825(a and b)

17 ☒ The following fees are submitted

## CALCULATIONS PTO USE ONLY

## BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

Search Report has been prepared by the EPO or JPO \$840.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)  
\$670.00No international preliminary examination fee paid to USPTO (37 CFR 1.482)  
but international search fee paid to USPTO (37 CFR 1.445(a)(2)) . . . \$760.00Neither international preliminary examination fee (37 CFR 1.482) nor  
international search fee (37 CFR 1.445(a)(2)) paid to USPTO . . . . . \$970.00International preliminary examination fee paid to USPTO (37 CFR 1.482)  
and all claims satisfied provisions of PCT Article 33(2)-(4) . . . . . \$ 96.00

840.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

\$ 840.00

Surcharge of \$130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30  
months from the earliest claimed priority date (37 CFR 1.492(e)).

\$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total claims	64 - 20 =	44	X\$18.00
Independent claims	7 - 3 =	4	X\$78.00

\$ 792.00

\$ 312.00

MULTIPLE DEPENDENT CLAIM(S) (if applicable) +\$260.00

\$ 260.00

TOTAL OF ABOVE CALCULATIONS =

\$2,204.00

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement  
must also be filed (Note 37 CFR 1.9, 1.27, 1.28).

\$

SUBTOTAL =

\$2,204.00

Processing fee of \$130.00 for furnishing the English translation later than ☐ 20 ☐ 30  
months from the earliest claimed priority date (37 CFR 1.492(f)).

\$

TOTAL NATIONAL FEE =

\$2,204.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be  
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +

\$

TOTAL FEES ENCLOSED =

\$2,204.00

Amount to be:  
refunded

\$

charged

\$

a. ☒ A check in the amount of \$ 2,204.00 to cover the above fees is enclosed.b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any  
overpayment to Deposit Account No. 13-4500. A duplicate copy of this sheet is enclosed.

Order No. 2026-4236US1

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR  
1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

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REGISTRATION NUMBER

09/242202

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PATENT

Docket No.: 2026-4236US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE (DO/EO)

Applicant(s) : Edward NELSON et al  
International  
Application No. : PCT/US97/14306  
International  
Filing Date : 14 August 1997 (14.08.97)  
Priority Date : 14 August 1996 (14.08.96)  
For : A NOVEL VECTOR FOR POLYNUCLEOTIDE  
VACCINES

Assistant Commissioner of Patents  
Box PCT  
Washington, D.C. 20231

Attention: DO/EO/US

PRELIMINARY AMENDMENT

Sir:

Prior to the examination on merit, Applicants are filing the following

Preliminary Amendment:

In the specification:

On page 13, line 26, delete [CGCIGTAGGT] and replace with  
-- CGCTGTAGGT --;

On page 13, line 27, delete [CCCCCGTIC] and replace with  
-- CCCCCGTTC --.

Please delete the sequence listing at pages 75-95 and insert the substitute  
sequence listing, pages 75-93 enclosed as Exhibit A.

661011 202211260

In the claims:

Please cancel claims 55-62 without prejudice.

REMARKS

Support for the amendment to the specification is on page 14, lines 17 and 18.

Similar correction has been made in SEQ. ID No: 1 in the enclosed substitute sequence listing.

With the cancellation of claims 55-62, claims 1-6, 8-30, 32-48, 50-62 and 64-69 are pending.

In compliance with the sequence listing rule, Applicants have filed herein a substitute paper sequence listing (Exhibit A) and computer readable sequence listing (Exhibit B). Applicants have also filed herein a Statement Under 37 C.F.R. §1.825(a and b) verifying that the substitute written sequence listing and computer readable sequence listing are identical and that no new matter has been added (Exhibit C).

Early and favorable action by the Examiner is earnestly solicited.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

Date: February 11, 1999

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09/242202

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## A VECTOR FOR POLYNUCLEOTIDE VACCINES

### Field of the Invention

The present invention relates generally to polynucleotide vaccines and a novel vector useful for same. The present invention relates more specifically to a humanized polynucleotide vector vaccine which is useful in generating an immune response to a selected target antigen, in particular, to a tumor antigen.

### Background of the Invention

Cancer is a leading cause of morbidity and mortality. Conventional therapies such as surgery, radiation treatment and the like have resulted in a modest improvement of survival. There remains a need in the art for alternative treatments. With the advancing knowledge of immunology and tumor interaction with cells of the immune system, the upregulation of the immune system provides an alternative approach to treatment of cancers.

The process of oncogenesis involves multiple steps and predominantly involves intracellular protein products of either oncogenies or tumor suppressor genes (3-7). These intracellular proteins frequently have mutations or alterations leading to dysregulation or altered activity which can result in 'altered self' protein sequences and therefore, potential tumor-specific antigens (8,9). The immune response to any tumor requires recognition of such tumor associated antigens. These more subtle, 'altered self', antigenic differences are likely to be much less immunodominant than truly foreign antigens such as those from infectious disease pathogens or allograft tissues. The recent work defining antigen presentation has revealed that antibodies generally recognize antigens in the form of whole molecules, T cells typically recognize antigens in the form of small peptides combined with MHC molecules. This understanding of antigen presentation has aided the recent identification of a number of tumor-associated antigens which are recognized by T cells and derived from intracellular proteins (9). The characterization of oncogene mutations, dysregulated developmental or oncofetal genes, and mutations of tumor suppressor genes provides multiple potential unique target antigens for anti-tumor immune

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responses.

One form of cancer, breast cancer is the most common cancer affecting women in the United States of America, with 183,400 new cases annually (1). Breast cancer is also the second most frequent single case of female cancer mortality with 46,000 deaths annually (1). Although there has been significant progress over the past several decades in the surgical, radiotherapeutic, and medical treatment of this disease, none of these modalities alone or in combination provide curative therapy for the majority of patients with advanced (stage III or metastatic) breast cancer. Less than 40% of patients with advanced breast carcinoma will be alive and disease free, five years from diagnosis and initial treatment (2). Interestingly, patients with significant inflammatory infiltrates in the primary tumor, i.e. medullary carcinoma, have significantly improved survival (2) despite a higher degree of cellular anaplasia. The recent identification of tumor suppressor gene loss, oncogene mutations and oncogene dysregulation in breast cancers has opened new horizons for the treatment of the extraordinarily common cancer.

Numerous oncogene alterations have been documented in patients with breast adenocarcinoma including p53, HER2/neu (C-erbB-2), Rb, ras, PEM/MUC 1, BRCA1, BRCA2, Int-2, and hst. These particular oncogene alterations yield a panel of potential tumor associated targets for immunomodulatory therapies. Up to 80% of advanced breast adenocarcinomas will have p53 alterations (10-14) with most of the mutations occurring in the so called "hot spot", between codons 130 and 286, (15-20). The C-erbB-2 or HER-2/neu gene product shares homology with the epidermal growth factor receptor (21-25) and is expressed in up to 40% breast carcinomas (26). The tumor suppressor gene Rb has been noted to be altered or absent in 24% to 42% of breast cancer patients (27-29) and this percentage appears to be increased in metastatic lesions over primary tumors. The role of ras alterations appears to be more complex in breast cancer than for most other tumors (30-33). H-ras is the member of this family of proteins which is most frequently altered in breast cancers although, either K ras and/or H ras are overexpressed in excess of 70% of breast cancers. Polymorphic Epithelial Mucin (PEM) or MUC-1 is a tumor-associated antigen which is now known to represent an underglycosylated and dysregulated glycoprotein (34-36) and is diffusely overexpressed on 94% of breast cancers (37). BRCA1 has recently

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been cloned (38) and mutations have been characterized in patients with ovarian and breast cancer (39-43). Int-2 and Hst are oncogene sequences encoding proteins which are members of the fibroblast growth factor (FGF) supergene family (44). Amplification of Int-2 and Hst is significantly correlated with invasive breast carcinoma (45, 46) and estrogen receptor positivity (47). Breast carcinomas have other alterations including overexpression of non-mutated ubiquitous cellular proteins such as c-myc, Bcl-1, Bcl-2, EGFR, PRAD1 (cyclin D1), IGF1R, bFGF, PDGF-B, TGF- $\beta$  and others (48, 49). When the novel or mutated regulatory elements responsible for the overexpression of these individual genes are identified and characterized they could prove to be appropriate target tumor antigens. Clearly, there is a diverse and growing set of potential tumor antigens as a result of the multiple molecular events inherent in the development of breast adenocarcinoma.

Evidence has recently accumulated suggesting a potential for immunotherapeutic approaches in the treatment of breast cancer. Patients with breast cancer can have a cellular response to autologous tumor associated antigens (50). Breast cancer patients can also have antibodies to mutated p53 proteins (51). Elution of antigens from immune complexes present in sera of patients with breast carcinoma reveal multiple oncogenic antigens (52). T-lymphocytes reactive to mutated p53, HER2/neu, and ras peptides have been described (9, 53, 54-58). PEM/MUC-1 has been shown to be recognized by both antibodies and cytotoxic T lymphocyte (CTL) lines (34, 52, 59-61). Some CTL recognize the target antigen in a non-MHC restricted fashion (52, 58-61) however, in a murine model classical HLA restricted CTL recognizing the non-repeating core sequences have been elicited (62, 63). These findings, in addition to the association of improved survival with inflammatory infiltration of primary breast tumors, suggest that patients can mount an immune response to the malignant cells of breast tumors.

Oncogene and tumor suppressor genes can have mutations within confined domains, such as p53 and ras (15-20, 64, 65), while others have mutations which inactivate the protein product and/or can have total loss of the normal alleles, such as RB-1 and BRCA1 (5-7, 66-70). Obviously, allelic deletions provide no protein product but, mutated inactive gene products can be the source of peptides recognized as tumor associated antigens. Significantly, humoral and CTL immune responses to

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polypeptides encompassing the mutated sequences for both p53 and ras have been identified (50-52, 55, 71, 72, 73). These genetic alterations from germline can be highly specific and unique for any one individual tumor, even those with identical phenotypic features, and may yield unique individual tumor antigens (74, 53, 17, 51). Some oncogenes products such as HER2/neu (C-erbB-2), PEM/MUC-1, Int-2, Hst-1, and TRE17 (75-80) do not display polymorphism due to mutations but rather, are overexpressed in malignant tissues and essentially not expressed in normal adult tissues. These proteins and glycoproteins which are preferentially expressed in/on neoplastic mammary cells more closely fit the classical model of tumor antigens. When immune responses have been demonstrated to these preferentially expressed proteins and responses are frequently limited to regions of dissimilarity with the homologous or normal proteins expressed in adult tissues (9, 32, 56, 58, 60). Therefore, regions of dissimilarity, like mutated sequences, are potential antigenic targets for immunomodulation of the anti-tumor immune response.

The ability to precisely define and characterize tumor associated antigens has facilitated the design and evaluation of immunomodulatory therapies. Effective immunotherapies have been largely limited to non-Hodgkin's lymphoma and melanoma. In the case of non-Hodgkin's lymphomas, the idiotypic immunoglobulin molecule has been a target of several different strategies (81-84). The use of anti-idiotypic antibodies resulted in some clinical responses but, modulation of the antigen was also seen (85, 86). Use of the intact idiotypic immunoglobulin molecule as a polypeptide vaccine has elicited both cellular and humoral immune responses which correlate with improved clinical courses (87-89). This suggests that even within a single tumor-associated protein a broader immune response, potentially to multiple epitopes within the polypeptide, will result in improved clinical efficacy. Several groups have conducted trials using allogeneic cells or allogeneic cellular preparations in an attempt to address the issue of antigenic heterogeneity within tumors but, with notable exceptions these strategies have not contributed significantly to improved survival in any tumor type (9, 90, 91). Although the evaluation of clinical efficacy rests on improved survival, immunological parameters may be surrogate end points which facilitate the design and evaluation of new strategies. In the case of these "polyvalent" preparations, the ability to adequately evaluate immunologic parameters



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is severely hindered by the lack of defined antigens. To date, there has not been an immunomodulatory trial with multiple, defined, autologous, tumor associated antigens.

Intratumoral heterogeneity dictates that antigen specific immunomodulatory maneuvers be individualized or face severe limitations on the patient population for which they are applicable. Single antigen based immunomodulatory strategies do not account for intratumoral heterogeneity and thus, are at risk for tumor escape from immune surveillance even if effective augmentation of the anti-tumor immune response is accomplished. Based on our current understanding of the biology of neoplasms, including the multitude of cellular processes which must be deranged for transformation and tumorigenesis, each patient's tumor offers multiple potential targets for antigen-specific, immunomodulatory therapies. A more effective and efficacious strategy should take advantage of this multiplicity of potential, highly specific, tumor antigens. However, there is no current immunomodulatory strategy which allows for this individualized 'polyclonal' approach.

We now appreciate that, as with the immune response in allograft rejection or viral infections, an effective anti-tumor immune response must involve a cytolytic cellular response. Evidence is accumulating that the MHC class I pathway of antigen presentation and cytotoxic T lymphocytes (CTL) are important for effective in vivo anti-tumor immune responses (62, 89, 92-96). It has been shown that even in vaccine studies using exogenous protein antigen, modest increases in the cytotoxic T lymphocyte compartment have been demonstrated and correlated with freedom from progression (89). Furthermore, the preeminent role of the TH1 phenotype of T helper cells in both humans and animal models supports the critical role of multiple arms of the cellular immune system for an effective anti-tumor immune response (97-101) and provides further evidence for the critical role of CTL anti-tumor immune responses.

The recently described polynucleotide vaccine strategy has been demonstrated to yield humoral and cellular (both helper and cytotoxic T lymphocyte) immune responses to numerous encoded antigens (102, 103, 104-131). This is in contrast to the overwhelming humoral response to exogenous protein or cellular vaccine preparations. This strategy uses covalent closed circular (CCC) plasmid DNA, 'naked DNA', to express the target antigen (117). These plasmids are non-replicating but, are capable of extended stable expression of the target sequences in skeletal muscle

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and potentially in professional antigen presenting cells such as members of the macrophage lineage or dendritic cells (117). The plasmids are non-replicating but, capable of stable mRNA expression within skeletal muscle and reticuloendothelial cells. Thus, antigens encoded by these mRNA sequences are processed as endogenous proteins and presented to the immune system in a manner analogous to presentation of virus encoded protein antigens. It is now recognized that most potential tumor antigens are recognized as peptides derived from intracellular proteins, some of which are dysregulated or mutated oncogene products (103, 132a, 133a, 134a, 104-112, 118, 119). Polynucleotide vaccine strategy provides an avenue for eliciting immune responses to such intracellular oncogene or dysregulated products. Multiple studies have demonstrated the expression of target antigens following intramuscular injection and uptake of 'naked DNA' (132a, 133a, 134a, 103, 1-20). The uptake of DNA is increased if preceded by 24 hours with an intramuscular injection of a myo-toxic agent in the same vicinity (133a, 134a, 1, 7, 10, 15).

The existing vectors use an antibiotic resistance gene as the selection agent. These genes generate a foreign protein, raise the possibility of an anti-vector immune response, and have the theoretical risk of transferring antibiotic resistance to normal host flora. These vectors use the CMV promoter, b actin promoter, or other retroviral LTR elements to drive transcription of the target sequence. These promoter elements have ubiquitous activity but, are large in the case of CMV and b actin, and in the case of the LTR have the theoretical risk of integration and potential oncogenic event. All of the current vectors are designed with a single target antigen in place with little or no flexibility for changing target sequences. The functional size limitation of all plasmid vectors limits the size of insertable elements. By utilizing large promoter elements and the complete ColE1 or MB1 origins of replication the size of the vector is increased and limitations are placed on the size and number of insertable elements. Thus, none of the existing vectors are optimally designed for evaluation of an anti-tumor polynucleotide vaccine strategy.

The majority of the antigens evaluated to date are generally monomorphic and thus, can be readily incorporated into established plasmid vectors. However, putative tumor antigens, i.e., altered self proteins, in any given tumor are not heterogeneous and in some cases distinctly unique. For example, mutations in p53, although limited

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to a contiguous region of approximately 600bp, are very heterogeneous from tumor to tumor (15-20). Other intracellular tumor antigens will likely demonstrate similar heterogeneity in the range of mutations. Therefore, the present invention is a vector which accommodates both monomorphic and polymorphic target antigens via PCR technology. This vector is useful in generating patient-specific, target-specific multiple antigen, anti-target polynucleotide vaccines.

### Summary of the Invention

An object of the invention is a humanized, polynucleotide vector.

An object of the invention is a kit comprising a humanized, polynucleotide vector alone, or in combination with at least one nucleic acid sequence encoding one or more target antigens or antigenic epitopes thereof.

Another object of the invention is a humanized polynucleotide vector vaccine useful in eliciting an immune response against one or more target antigens or antigenic epitope thereof.

Yet another object of the invention is a pharmaceutical composition comprising the humanized polynucleotide vector vaccine and a pharmaceutically acceptable carrier.

A further object of the invention is a kit comprising a humanized polynucleotide vector vaccine alone or in combination with an expression enhancing agent.

Another object of the invention is a sequence acceptance site which accepts cDNA target products from rt-PCR cloning.

One aspect of the invention is a method of preparing a humanized polynucleotide vector.

Another aspect of the invention is a method of preparing a humanized polynucleotide vector vaccine.

Yet another aspect of the invention is a method for expressing at least one target antigen or antigenic epitope thereof in cells comprising introducing a humanized polynucleotide vector into cells under conditions for expression of the target antigen or antigenic epitope thereof.

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Another object of the invention is a host cell expressing at least one target antigen or antigenic epitope thereof provided by a polynucleotide vector.

A further aspect of the invention is a method of stimulating a specific immune response to at least one target antigen or antigenic epitope thereof in a mammal comprising the administration of an effective amount of a polynucleotide vector vaccine into the mammal to elicit the specific immune response.

Another aspect of the invention are target antigen specific monoclonal and polyclonal antibodies elicited by administration of a polynucleotide vector vaccine.

Yet another aspect of the invention are target antigen specific cytotoxic lymphocytes and target antigen specific helper T lymphocytes elicited by administration of a polynucleotide vector vaccine.

These and other objects, features and many of the attendant advantages of the invention will be better understood upon a reading of the detailed description of the invention and drawings.

### Brief Description of the Drawings

Figure 1 is a genetic map of the polynucleotide vector, pITL.

Figure 2 depicts the insertion site in the polynucleotide vector, pITL, for cDNA encoding target antigen.

Figures 3A through 3C show the amino acid sequence homology between human epidermal growth factor receptor (EGFR) and human Her 2/neu. The line underscores the target sequence of human Her 2/neu. The bold letters indicate the transmembrane domain.

Figures 4A through 4C show the amino acid sequence of human Her 2/neu and Rat Her 2/neu. The lines overlies the target sequence for both human and rat Her 2/neu. The bold letters indicate the transmembrane domain.

Figure 5 shows the restriction enzyme sites of the polynucleotide vector pITL.

### Detailed Description Of The Invention

The present invention is a "humanized" vector which has the necessary elements to express mRNA for a target antigen. The resultant translated polypeptides are available for processing into presentable antigens to the immune system. The vector accommodates monomorphic and polymorphic nucleic acid sequences of a

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target antigen or antigens. The vector of the present invention is useful for constructing polynucleotide vector vaccines or "naked DNA" vaccines containing a nucleic acid sequence encoding one or more target antigens.

The polynucleotide vaccines vector of the present invention has the following characteristics:

- (1) is selectable for growth and production of a polynucleotide vaccine product,
- (2) is capable of eukaryotic expression of one or more target antigens or antigenic epitopes thereof,
- (3) is functional in selected tissue and selected cells of the inflammatory immune system,
- (4) has minimal extraneous non-human DNA sequences to minimize potential toxicity, and
- (5) is capable of accepting target nucleic acid sequences from a number of different transcripts.

A polynucleotide vaccine of the invention offers multiple advantages over other vaccine strategies for immunomodulation. Polynucleotide vaccine strategies appear to elicit brisk CTL responses in all models in which this aspect of the immune response has been evaluated as seen in Table 1. The primary advantage of the polynucleotide vaccine of the invention is that the target antigen(s) is expressed as an intracellular polypeptide or peptide and, as such, is processed as a self polypeptide or peptide and appropriately presented on antigen presenting cells.

TABLE 1

ANTIGEN	MODEL SPECIES	IMMUNE RESPONSES			TH1/TH2	FUNCTIONAL EFFECT
		HUMORAL	PROLIFERATIVE	CTL		
Rabies	Mouse	+		+	TH1	Protective
Plasmodium	Mouse	+		+		Protective
Influenza, Nucleoprotein	Mouse	+		+		Protective
	Mouse			+		
Influenza, Hemagglutinin	Mouse	+				Protective
	Mouse	+				Protective
	Chicken	+				Protective
	Chicken	+				
	Mouse	+		+		
Bovine Herpes Virus - 1	Bovine	+				Decreased Shedding
	Mouse	+				Protective & Ig Class Switch
HBV, Envelope	Mouse	+				
HBV, Surface A	Rabbit	+				
HCV, Core Protein	Mouse	+	+	+		
HCV, Nucleocapsid	Mouse	+				
HSV, Glycoprotein D2	Guinea Pig	+				Protective
HIV-1, GP160	Mouse	+	+			Reduced Syncytia
	Mouse	+		+		
HIV-1, GP160	Primate	+				Reduced Syncytia
HIV-1, GP120	Primate	+				

SIV	Primate	+		+		protective
BBTA GAL	Primate	+				Expression
Luciferase	Primate	+				Expression
Apolipoprotein E	Rat					Expression
CEA	Mouse	+		+		Protective
p53	Mouse	+				
Factor IX	Mouse	+		+		Expression
h Growth Hormone	Mouse					Expression
Dystrophin	Mouse	+				Expression

SIV	Primate	+		+		Protective
BBTA GAL	Primate	+				Expression
Luciferase	Primate	+				Expression
Apolipoprotein E	Rat			+		Expression
CEA	Mouse	+				Protective
p53	Mouse	+			+	
Factor IX	Mouse	+				Expression
h Growth Hormone	Mouse					Expression
Dystrophin	Mouse	+				Expression

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Other added advantages of the polynucleotide vector as a vaccine is that preparation of plasmid DNA is much less labor and time intensive than cellular or protein vaccine preparations. Polynucleotide vaccine preparations are readily purified, sterilized, and DNA preparations are known to have a long shelf life when stored as a precipitate. Technical expertise in standard sterile tissue handling procedures, standard molecular techniques, and established PCR technology is all that is required for generation of the polynucleotide vaccine preparation. Administration is by standard medical procedures performed routinely. Safety concerns are minimal as no retroviral or oncogenic viral elements are used in the present invention. Finally, with the vector of the present invention, the only protein to be transcribed and translated is the target antigen thus, unlike the pox virus vectors or existing polynucleotide vaccine vectors there is no acquired immunity to the vector of the present invention.

The polynucleotide vector of the present invention has minimal exogenous, non-human DNA and has been maximally "humanized". A 'humanized' vector is one in which the majority of the functional elements are derived from or synthesized based on a human element or a mammalian homolog of the human element. The intent of humanization is to achieve sustained expression of a target antigen(s) with no or minimal risk of methylation and genetic downregulation which would prevent or inhibit translation of the target antigen(s). Therefore, the vector does not contain sequences which may increase inactivation by methylation or changes in tertiary structure. Thus, the polynucleotide vector selectively elicits immune responses to the target sequence(s) with little or no immune response to the other components of the polynucleotide vector.

The humanized polynucleotide vector comprises a human derived promoter or mammalian homolog thereof which is functional in a mammalian target tissue and mammalian target cells and a sequence acceptance site which accepts cDNA target products from rtPCR cloning.

There are minimal non-human components in the polynucleotide vector. These components of the polynucleotide vector which are non-human derived components are necessary for production of the vector. These non-human components are the origin of replication which allows replication and growth of the vector in bacterial or yeast host cells and a nucleic acid sequence which allows for selection of recombinant



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plasmids in the bacterial or yeast host cells.

In one embodiment, the plasmid comprises an origin for plasmid replication and growth in bacterial cells, a nucleic acid sequence which allows for selection of recombinant plasmids which is operably linked to a human-derived promoter which is functional in a mammalian target tissue and mammalian target cells, a cloning site for insertion of sequences encoding target antigen(s), a stuffer sequence, a human derived 3' splice sequence, and a human derived poly A sequence.

The origin of replication may be non-human and may be derived from several sources including bacteria and yeast and the like. Such origins of replication include, but are not limited to PMB1, pUB110, pBC16, pSAQS01, pX012, pE194, pC194, pS174pSA2100, pSE3, pAM330, pCG1, pCG4, pHM1519, pSa151, pLS103, pTA1060, pBs81/6, pSC101, R1, RK2, RSF1010, ARS1, R6K, Sa, R300B, Rep, RepA, pRO1614, OriT, OriV, OriW, OriC, OriF, OriP, OriT, OriT, CdE1, pEW27, pE194, fd, F1, F, NR1, p15A, a colE1 origin, functional portions thereof, and the like which are available from plasmids deposited with the American Type Culture Collection (ATCC), Rockville, MD. In a preferred embodiment, the origin of replication is a colE1 origin or functional portion thereof. In one embodiment, the origin of replication for use in constructing the polynucleotide vector is a minimal colE1 origin isolated from the vector pBR327 (ATCC) (Oka, A. et al Molec. Gen. Genet. Vol. 172, 151-159, 1979) comprising the sequence:

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GGCCGCGTTG CTGGCGTTTT TCCATAGGCT CCGCCCCCCT GACGAGCATC
ACAAAAATCG ACGCTCAAGT CAGAGGTGGC GAAACCCGAC AGGACTATAA
AGATACCAGG CGTTTCCCCC TGGAAGCTCC CTCGTGCGCT CTCCTGTTCC
GACCCTGCCG CTTACCGGAT ACCTCTCCGC CTTTCTCCCT TCGGGAAGCG
TGGCGCTTTC TCAATGCTCA CGCIGTAGGT ATCTCAGTTC GGTGTAGGTC
GTTTCGCTCCA AGCTGGGCTG TGTGCACGAA CCCCCCGTIC AGCCCGACCG
CTGCGCCTTA TCCGCTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG
ACTTATCGCC ACTGGCAGCA GCCACTGGTA ACAGGATTAG CAGAGCGAGG
TATGTAGGCG GTGCTACAGA GTTCTTGAAG TGGTGGCCTA ACTACGGCTA
CAC (SEQ. ID. NO. 1), and analogs thereof.
```

The minimal colE1 origin is a minimal size yet functional and provides plasmid replication and growth within permissive strains of E. coli. The colE1 in antiparallel as it reads in the polynucleotide vector and comprises the sequence:

```
GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC
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ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC AGTGGCGATA  
AGTCGTGTCT TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG  
CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA GCTTGGAGCG  
AACGACCTAC ACCGAACTGA GATACCTACA CCGTGAGCAT TGAGAAAGCG  
CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG  
GTCCGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA  
TCTTTATAGT CCTGTGCGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT  
TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG  
GCC (SEQ. ID. NO. 2), and analogs thereof.

In a preferred embodiment, the *colE1* origin of replication in the polynucleotide vector comprises the sequence:

CTCGGGCCGCGTTGCTGGCGTTTTCATAGGCTCCGCCCCCTGACGAG  
CATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGAC  
TATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCT  
GTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGA  
AGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTA  
GGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCCG  
ACCGCTGCGCCTTATCCGGTAACCTATCGTCTTGAGTCCAACCCGGTAAGA  
CACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGC  
GAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACG  
GCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTT  
ACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGC  
TGGTAGCGGTGGTTTTTTTGTITGCAAGCAGCAGATTACGCGCAGAAAAA  
AAGGATCT (SEQ. ID. NO. 26)

This fragment of the *colE1* replicon was isolated by digesting pBR327 with Bst Y1 and Ava I. After isolation of the fragment by agarose gel electrophoresis the ends were polished to blunt with klenow fragment (large fragment) of DNA polymerase I. Then this fragment was ligated into pBluescript SK at the Sma I site and orientation was determined such that the orientation of the replicon was anti-parallel to the origin of replication of the pBluescript. The SupF fragment was isolated and ligated as described herein, orientation was again determined such that the SupF was read in an anti-parallel direction to the RANTES promoter and the putative target sequence in the completed vector.

A sequence which will provide a mechanism for selection and growth of recombinant plasmids in bacteria or yeast is provided in the vector construct. However, the polynucleotide vectors does not contain foreign antibiotic resistance genes. The sequences may be non-human derived sequences. The polynucleotide vector uses sequences such as suppressor tRNA genes including but not limited to as

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SupE, SupP, SupD, SupU, SupF, SupZ, glyT, glyU, SerP,  $psu_1^+$ ,  $psu_2^+$ -C34,  $psu_3^+$ am,  $psu_3^+$ OC (Eggertson, G. et al, Am. Soc. Microbiology, vol. 52:354-374, 1988), and synthetic supF complementation tRNA gene, derivatives thereof (Brown, El. et al. Methods in Enzymology Vol. 68:109-51, 1979) and the like to provide a mechanism for selection and growth.

The synthetic supF complementation tRNA gene (143) provides the mechanism for selection and growth of recombinant plasmids in a manner analogous to that used in eukaryotic expression cloning using the 'Seed' vector (pCDM8 or derivatives). This selection is dependent upon the presence of the 60kb p3 helper plasmid which contains inactive tetracycline and ampicillin resistance genes due to amber stop codon mutations which are complimented by the supF tRNA. The p3 helper plasmid is derived from pLM-2 as described in Mindich, L. et al. J. Bacteriology vol. 126, pp. 177-182, 1976. The supF tRNA is not functional in eukaryotic cells.

In one embodiment, a Sup F gene sequence for use in the polynucleotide vector comprises:

```
GAATTCTTTC GGACTTTTGA AAGTGATGGT GGTGGCCGAA GGATTCGAAC
CTTCGAAGTC GATGACGGCA GATTTAGAGT CTGCTCCCTT TGGCCGCTCG
GGAACCCAC CACGGGTAAT GCTTTTACTG GCCTGCTCCC TTATCGGGAA
GCGGGGCGCA TCATATCAAA TGACGCGCCG CTGTAAAGTG TTACGTTGAG
AAAGAATTC (SEQ. ID. NO. 3), and analogs thereof.
```

The synthetic supF complementation tRNA gene may be isolated from the vector, pVX, which is present in the bacterial strain 39083 available at the American Type Culture Collection. The Sup F sequence as it reads in the polynucleotide vector is antiparallel and comprises:

```
1 GAATTCTTTC TCAACGTAAC ACTTTACAGC GGCGCGTCAT TTGATATGAT
  GCGCCCCGCT TCCCGATAAG GGAGCAGGCC AGTAAAAGCA TTACCCGTGG
  TGGGGTTCCC GAGCGGCCAA AGGGAGCAGA CTCTAAATCT GCCGTCATCG
  ACTTCGAAGG TTCGAATCCT TCCCCACCA CCATCACTTT CAAAAGTCCG
30 AAAGAATTC (SEQ. ID. NO. 4), and analogs thereof.
```

Promoters for use in the vector are human promoters or functional portions thereof, promoters derived from or synthesized based on a human promoter, and mammalian homologs of human promoters, or portions thereof. The promoters for use in the polynucleotide vector of the present invention do not encompass viral promoters or viral derived promoters. The human promoters are selected on the basis

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of the tissue and cells to be targeted and should provide optimal expression of the target antigen(s) or antigenic epitopes thereof in the selected mammalian target tissue and mammalian cells. Embodiments of promoters which may be used in constructing the polynucleotide vector of the present invention include but are not limited to the human derived RANTES promoter (Nelson, P.J. et al. J. Immunol. Vol. 151:2601-33, 1993; Ortiz; B.D. et al. Molecular Cell. Biol. pp 202-210, 1996 (Jan); Nelson, P.J. Immunol Vol. 157, No. 3, 1996 (August 1)), truncated RANTES promoters and derivatives thereof. Truncated RANTES promoters include but are not limited to a 249 base pair fragment, a 440 base pair fragment and a 900 base pair fragment. In a preferred embodiment, the truncated RANTES promoter is a base pair fragment of approximately 440 base pairs. A preferred truncated RANTES promoter is a fragment that spans the region approximately between the NCO restriction endonuclease site through the KpnI site of the RANTES promoter as described in GenBank Accession No. S64885. The human derived promoter is selected based on the mammalian target tissue and mammalian target cell in which expression of the target antigen(s) is desired. The target tissue is one containing antigen presenting cells or easily accessible to antigen presenting cells. Such tissue include but is not limited to muscle, lymph nodes, epithelium, subepithelium, skin, and the like. In one embodiment, the promoter is active in muscle or skin. Promoters may express one or more target antigen(s) or antigenic epitope thereof in muscles cells or professional antigen presenting cells. Preferred are promoters which express target antigen(s) in professional antigen presenting cells such as monocytes, macrophages, dendritic cells, Langerhans cells and the like.

The 3' splice sequence and the poly A sequences in the polynucleotide vector are a mammalian sequence, or a synthetic sequence based on a mammalian sequence, preferably a human sequence or mammalian homolog thereof. Possible sources of these elements include but are not limited to bovine growth hormone, human growth hormone gene, and the like. In a preferred embodiment of the polynucleotide vectors the 3' splice (intron) and the poly A sequences are synthetic sequences based on sequences from the human growth hormone gene (DeNoto, F.M. et al. Nuc. Acids. Res. Vol. 9(15):371930, 1981). Oligonucleotides may be synthesized based on the published sequence or modified to condense the third intron and poly A tail sequences

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along with the appropriate splice functions. Poly A-signal sequences which may be used in constructing the polynucleotide vector include but are not limited to:

AATAAA (SEQ. ID. NO. 5)  
 ATTAAA (SEQ. ID. NO. 6)  
 AGTAAA (SEQ. ID. NO. 7)  
 AAGAAC (SEQ. ID. NO. 8)  
 AATACA (SEQ. ID. NO. 9)

and the like.

In one embodiment, a combined 3' splice sequence and poly A tail sequence for incorporation into the polynucleotide vector construct includes but is not limited to:

5' GCCTTAAGGG CCATATGGTG AGTGGATCCC TTGACCCCAG GCGGGGATGG  
 3' GGAATTCCC GGTATACCAC TCACCTACGG AACTGGGGTC CGCCCTACC  
 GGAGACCTG TAGTCAGAGC CCCCAGGCAG CACAGGCCAA TGCCCGTCCT  
 CCCTCTGGAC ATCAGTCTCG GGGGCCCGTC GTGTCCGGTT ACGGGCAGGA  
 TCCCCTGCAG GATGAGTAGT GAGTGCCTCT CCTGGCCCTG GAAGTTGCCA  
 AGGGGACGTC CTACTCATCA CTCACGGAGA GGACCGGGAC CTTCAACGGT  
 CTCCAGTGCC CACCAGCCTT GTCCTAATAA AATTAAGTTG CATCATTTTG  
 GAGGTCACGG GTGGTCGGAA CAGGATTATT TTAATTCAAC GTAGTAAAAC  
 TCTGACTAGG TGTCCTCTAT AATATTAT 3' (SEQ. ID. NO. 10)  
 AGACTGATCC ACAGGAGATA TTATAATA 5' (SEQ. ID. NO. 11)

Operably located downstream of the promoter and upstream of the splice and poly A sequences, is the cloning site and stuffer site. The sequence acceptance site is functional in mammalian cells, preferably human cells. The sequence acceptance site is synthetically constructed, however, the sequences are derived from sequences that function in human cells. The sequence acceptance site is designed to directionally accept sequence-specific products from rtPCR-based cloning strategies via unique sequences within the interrupted palindrome recognition sequence for the Bgl I restriction endonuclease, which is incorporated into the PCR primers. The 5' cloning site of the vector was designed in such a way as to provide an integral Kozak consensus sequence (145) and an in-frame initiation codon. Oligonucleotides may be synthesized for the sequence acceptance site so as to provide an initiation codon,

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Kozak consensus sequence and downstream termination codon. Kozak consensus sequence which may be incorporated into the polynucleotide vector includes but is not limited to:

GCCCGCC (either A or G) CCAUGG and the like, as are known in the art (Kozak, M. J. Cell Biol. vol. 108, pp 229-241, 1989).

The polynucleotide vector may be constructed to allow for insertion of a internal ribosomal entry site (IRES). Such a site allows for translation of a second open reading frame from a polycistronic mRNA molecule. Such IRES elements are described by Sachs et al., Cell June 13, 1997 Vol. 89: 831-838; Chen, C-Y et al. Science Vol. 268, pp. 415-417, 1995; Tahara, H. et al. J Immunol pp. 6467-6474, 1995; Joshi, C. et al. Nucleic Acids Research Vol. 23, No. 4, pp. 541-549, 1995; Ting, J. et al. DNA Vol. 7, No. 4, pp 275-286, 1988; and Aharon, T. et al. Molecular Cellular Biology, Mar 1993, pp 1971-1980. A polynucleotide vector comprising an IRES allows for translation of more than one target antigen or epitope from the polycistronic mRNA. A polynucleotide vector comprising an IRES allows expression of a target antigen and a cytokine or chemokine from a polycistronic mRNA.

A stuffer site is provided in the vector as a space occupying, non-coding fragment of DNA. A stuffer site may be synthetically synthesized. In one embodiment the stuffer site has the nucleic acid sequence:

CCTCGGTACCTGCCATGGCGCGGATTCTTTATCACTGATAAGTTGGTGGA  
CATATTATGTTTATCAGTGATAAAGTGTCAAGCATGACAAAGTTGCAGCC  
GAATACAGTGATCCGTGCCGGCCCTGGACTGTTGAACGAGGTCGGCGTAG  
ACGGTCTGACGACACGCAAAGTGGCGGAACGGTTGGGGGTGCAGCAGCC  
GGCGCTTTACTGGCACTTCAGGAACAAGCGGGCGCCTTAAGGGCCATATG  
CCG (SEQ. ID. NO. 12), and variants thereof.

This stuffer region sequence is based on the stuffer region in vector pCDM8 (In Vitrogen). The sequence of the stuffer is not critical as long as it is one which does not interfere with cloning of the rt-PCR target sequence and does not contain a BglII site. The stuffer site is not present in the final polynucleotide vector vaccine, as it is excised.

In one embodiment of the present invention the polynucleotide vector

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comprises pITL as schematically depicted in Figure 1. cDNA encoding one or more target antigen(s) or antigenic epitopes thereof may be ligated into the cloning site or sequence acceptance site depicted in Figure 1. The sequence acceptance site is designed to directionally accept sequence specific products from rtPCR based cloning strategies via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease which is incorporated into the PCR primer. In one embodiment, the pallidrome recognition sequence is for the Bgl I restriction endonuclease. In a preferred embodiment the sequence acceptance site is as depicted in Figure 2 in which the 5' acceptance site reads on the (+) strand as the sequence GCCA/CCATGGCc wherein the GCC sequence is recognized by Bgl I and ATG is the start codon. GCc keeps the sequence in-frame and encodes the amino acid serine. The 3' acceptance site reads on the (+) strand as GCCTTAAGGGC.

Any exogenous gene may be inserted into the polynucleotide vector and expressed in a host cell or host tissue. Of interest are exogenous genes which are associated with diseases or pathological conditions in humans. Of particular interest are the antigens or antigenic epitopes thereof which are useful in stimulating an immune response in a mammal, preferably a human for the purpose of prevention or treatment of a disease or pathological condition.

The polynucleotide vaccine vector is useful as a single target antigen vaccine and as a multi-target antigen vaccine. Of interest are target antigens derived from intracellular organisms and the host intracellular compartment. The target antigen includes but is not limited to tumor antigen, bacterial, viral, parasitic antigen and the like. Such antigens include but are not limited to antigens or antigenic epitopes derived from rabies virus, plasmodium, Herpes Virus, HIV 1, HIV 2, influenza virus, HBV, HCV, SIV, Cytomegalovirus, Mycobacterium, Measles virus, papillomavirus, and the like. The polynucleotide vector is particularly well suited for expression of tumor associated genetic derangements, which encompass aberrant transcription regulatory controls on oncogene mutation, a dysregulated developmental or oncofetal gene, a mutated tumor suppressor gene, dysregulated cellular enzymes coding sequences such as metaloproteases or combinations thereof. Tumor antigen or antigenic epitopes thereof which may be expressed by the polynucleotide vector include but are not limited to p53, RB, ras, int-2, Hst, Tre17, BRCA-1, BRCA-2,

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MUC-1, HER-2/neu, PEM/MUC-1, and the like. The polynucleotide vector is also well suited for insertion of one or more target antigens or antigenic epitopes thereof derived for an individual's tumor. The nucleic acid sequences encoding one or more target antigens is ligated into the cloning site of the polynucleotide vector thereby forming the polynucleotide vector vaccine.

The nucleic acid sequence encoding a cytokine and/or chemokine may also be incorporated into the polynucleotide vector vaccine for enhancement of an immune response. Such cytokines include but are not limited to interleukin 2, interleukin 4, interleukin 7, granulocyte-monocyte colony stimulating factor (GM-CSF), tumor necrosis factor (TNF), interferon, interleukin 12, interleukin 3, interleukin 15, interleukin 8, interleukin 18 and the like. Nucleic acid sequences encoding chemokines which may incorporate into the cloning site of the polynucleotide vector vaccine include those that recruit lymphocytes and antigen presenting cells into a target tissue. These include but are not limited to RANTES, MCP, MIP- $\alpha$ , MIP-1 $\beta$ , defensins and the like.

In one embodiment, the polynucleotide vector vaccine comprises a gene encoding a target antigen or epitope thereof and a gene encoding GM-CSF or functional portion thereof. The polynucleotide vector allows expression of both the target antigen or epitope thereof and GM-CSF in the target cell or target tissue resulting in an enhanced immune response to the target antigen or epitope thereof.

The present invention encompasses methods of making a humanized polynucleotide vector which comprises operably linking an origin of replication with a nucleotide sequence which provides a mechanism for selection, which in turn is operably linked to a human derived promoter or mammalian homolog thereof which is functional in a mammalian target tissue and mammalian target cells. The promoter is operably linked with a cloning site containing a sequence acceptance site which directionally accepts target sequence specific products from rtPCR cloning. The 5' sequence acceptance site comprises an initiation codon and Kozak consensus sequence. The 3' sequence acceptance site comprises termination codons (Figure 2). The method also provides a stuffer region, two separate in1 frame termination codons, a human derived 3' splice (intron) or mammalian homolog thereof and a human derived poly A sequence.



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The present invention also provides a method of preparing a polynucleotide vector vaccine in which one or more nucleic acid sequences encoding one or more target antigens or antigenic epitopes thereof are incorporated. The method of making the substantially humanized polynucleotide vector vaccine provides a product able to express stable mRNA for one or more target sequences.

For vaccination against a tumor in a patient, the construction of the polynucleotide vector vaccine is tailored to the individual's tumor antigen or antigens. Most potential tumor antigens are recognized as peptides derived from intracellular proteins, some of which are dysregulated or mutated oncogene products (74, 8, 9, 30, 53, 71, 132b, 133b, 134b, 135b, 136, 138) as opposed to unique, intact cell surface molecules. Therapies such as monoclonal antibodies or peptide vaccine strategies are generally directed against a single tumor associated antigen and as such are confronted by the fundamental issue of intratumoral antigenic heterogeneity and are limited in their scope of applicability due to both intertumoral antigenic heterogeneity and the nature of most tumor-associated antigens. The multiple and diverse set of molecular events involved in neoplastic transformation, in the evolution to a malignant/metastatic phenotype, and in resistance to therapy provides a foundation for devising novel therapeutic strategies as each molecular event has the potential to generate a tumor specific antigen (139-141). Current molecular techniques and the recently described polynucleotide vaccine strategy (102, 132a, 133a, 134a, 135a, 103) allow a novel antigen-specific, anti-tumor, immunomodulatory therapeutic strategy. Polynucleotide vaccine strategies have been shown in animal models to elicit a broad antigen-specific immune response including humoral, proliferative and cytolytic T cell responses. The present invention addresses the issues of tumor heterogeneity and takes advantage of this phenomena to generate a highly specific yet broad anti-tumor immune response directed against multiple defined tumor-specific antigens.

Using RT-PCR technology, it is possible to isolate target cDNA sequences encoding antigenic polypeptides from individual tumors and to use this nucleic acid template in the polynucleotide vector to induce an immune response to the uniquely mutated or dysregulated intracellular tumor associated proteins in individuals immunized with the vaccine.

mRNA isolated from a patient's tumor is subjected to rtPCR by standard

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techniques. PCR primers are used to amplify, clone into the vector of the present invention and subsequently evaluate, by thermocycle sequencing, the resultant cDNA for mutations. If mutated sequences are identified and present in at least about 20% of clones analyzed, the sequences are used as a component of the polynucleotide vaccine of the present invention. For the oncofetal proteins, such as int-2 and Hst, demonstration of the presence of mRNA is sufficient for constructs with the oncofetal sequences to form part of the polynucleotide vaccine. Tumors which express MUC-1 or HER-2/neu antigens by immunohistochemistry have monomorphic constructs for these two tumor antigens which are ligated into the polynucleotide vector. Thus, each individual patient may receive a unique set of plasmid constructs depending upon the repertoire of mutated oncogenies, mutated tumor suppressor genes, expressed oncofetal genes, and disregulated monomorphic antigens. Examples of such include but are not limited to nucleic acid sequences for c-erb-  $\beta$ -2/HER2/neu, PEM/MUC-1, Int-2, Hst, BRCA-1, BRCA-2, truncated EGFRvIII, MUC-1, CEA, p53, ras, RK, Myc, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAP, MEN-1, ERB-B1 (Cell vol. 64:pp235-326, Jan. 25, 1991) and the like, which are inserted into the polynucleotide vector.

In one embodiment, the polynucleotide vector vaccine of the present invention include but are not limited to: pITL-HER2/neu, pITL-PEM/MUC-1, pITL-Int-2, pITL-Hst, pITL-BRCA-1, pITL-BRCA-2, pITL-p53, pITL-ras, pITL-RB, pITL-TRE17 and the like.

The polynucleotide vector vaccine of the invention may be formulated into a pharmaceutical composition comprising the vector vaccine and a pharmaceutically acceptable carrier such as physiological saline and the like, as known in the formulation art. The pharmaceutical composition may comprise one or more different polynucleotide vectors depending on the needs of the patient to be treated. The pharmaceutical composition may also comprise an agent which enhances the uptake and expression of the polynucleotide vaccine into the target tissue and target cells. These agents include but are not limited to mycotoxic agents. Such mycotoxic agents include but are not limited to dextrose, bupivacaine HCl (1-butyl-N-[2,6-dimethyl-phenyl]-2-piperidine carboxamide) (Sigma, St. Louis, MO), and the like.

The present invention also encompasses methods of stimulating an immune

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response, cellular and humoral, against a target antigen or antigenic epitope with little or no toxicity or autoimmune reactions using the polynucleotide vector vaccine. In the case where the target antigen is a tumor antigen or antigens, the immune response generated by the method should result in generation of cytotoxic T lymphocytes which are capable of inhibiting or killing tumors cells or premalignant cells expressing the tumor antigen(s).

In the method of treatment, at least one polynucleotide vector vaccine comprising a nucleotide sequence of the target antigen or multiple antigens is administered to a patient in a dose of at least about 1  $\mu$ g per component, preferably at doses of about 1  $\mu$ g to about 1 mg per DNA per construct, although higher or lower doses may be used. The dose is administered intramuscularly (IM) or by cutaneous ballistic impregnation in a volume of about 0.1 ml to a volume of about 1 ml. Preferably the volume is about 0.5 ml per injection. In one embodiment, the dose of vaccine is administered at three week intervals for a total of three doses.

In one embodiment of the method of treatment, each intramuscular injection of the polynucleotide vaccine is preceded by IM injection of an expression enhancing agent approximately 24 hours before vaccine administration. The enhancing agent may be bupivacaine-HCl, dextrose, and the like. In the case of dextrose, a concentration of about 10 to about 50% is administered, preferably a concentration of about 50%. Preferably the enhancing agent is bupivacaine-HCl and functional equivalents. Bupivacaine-HCl is administered in a concentration of about 0.25% to about 1.0%, preferably a concentration of about 0.5% in a volume of 0.5 ml.

In another embodiment of the method of treatment, the polynucleotide vector vaccine is administered using gene gun techniques. In this embodiment, the polynucleotide vector vaccine is adsorbed on to particles and administered by bombardment of the particles into the target tissue such as skin. (Yang, N.S., et al. 1990, Proc. Nat'l. Acad. Sci. USA 87: 9568; Williams, R.S. et al. 1991, Proc. Nat'l. Acad. Sci. USA 88:2726; Fynan, E.R.G. et al. Proc. Nat'l Acad. Sci. USA 90:11478; Eisenbraun, M.D. et al. DNA and Cell Bio. 1993 12:791).

Patients may also receive conventional cancer therapy prior to the polynucleotide vaccine therapy. Treatments that induce or result in immunodepression are discontinued a minimum of 4 weeks prior to immunization with the polynucleotide

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vaccine. In the case of viral, bacterial, yeast, parasitic infections, appropriate antiviral, antibiotics, antifungals and the like may be administered prior to, concurrent with, or after administration of the polynucleotide vaccine.

Patients are monitored at periodic intervals to assess the efficacy of the treatment. Tumor burden of the individual is monitored. The immune response to each component of each individual's vaccines preparation is evaluated. This is accomplished by evaluating the proliferative and cytolytic response to autologous cells transfected with single individual components (plasmid constructs). Tumor specific CTLp frequency is assessed for each patient. Anti-tumor humoral responses are assayed by staining of autologous tumor cells and analysis by fluorescent cell scanning (FACS) or the whole cell ELISA technique. Immune responses induced by polynucleotide vector vaccines containing nucleic acid sequences encoding virus, bacterial, yeast, parasite target antigens may be measured using standard immunoassays as are known in the art.

Polynucleotide vaccine therapy is applicable to any cancer in which a genetic defect is identified. Such cancers include but are not limited to breast cancer, prostate cancer, lung cancer, liver cancer, melanoma, colorectal cancer, pancreatic cancer, thymoma, sarcoma, non-Hodgkins lymphoma, Hodgkins lymphoma, leukemia, ovarian cancer and the like. Of particular interest is breast adenocarcinoma.

The invention further comprises an isolated antibody or antigen binding site thereof elicited by immunization with the polynucleotide vector vaccine. The antibody has specificity for and reacts or binds with the target antigen or antigenic epitope thereof. The antibody may be polyclonal, monoclonal, chimeric antibody or may be single chain antibody produced by genetic engineering. (U.S. Patent No. 4,946,778; Milenic et al Cancer Res. Vol 51:6363-6371, 1991; Shu et al Proc. Nat'l Acad. Sci USA, Vol. 90:7995-7999, 1993; Bird et al Science, Vol. 242:423-426, 1988; Traunecker et al The EMBO J., Vol. 10(12):3655-3659, 1991; Oi et al BioTechniques, Vol. 4, No. 3:214-221, 1986). Monoclonal antibody may be produced by *in vitro* or *in vivo* target antigen stimulation. In one embodiment, target antigen specific B lymphocytes are isolated from a human immunized with a polynucleotide vector vaccine comprising a sequence encoding the target antigen. Human hybridomas are produced by methods known in the art and the monoclonal

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antibody isolated and purified. (Cairns et al J. Clin. Invest., Vol. 74:880-887, 1984; Nakamura et al J. Immunol., Vol. 141, No. 12:4165-4172, 1988).

The antibody and antigen binding site thereof is useful as a therapeutic and also as a diagnostic agent to detect target antigen or antigenic epitope thereof in tissue and biological samples using standard immunoassays. (Yokota, et al Cancer Res., Vol. 52:3402-3408, 1992; Colcher et al Cancer Res., Vol. 48:4597-4603, 1988).

While the invention is described above in relation to certain specific embodiments, it will be understood that many variations are possible, and that alternative materials and reagents can be used without departing from the invention. In some cases such variations and substitutions may require some experimentation, but will only involve routine testing.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept, and therefore such adaptations and modifications are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments.

All references and patents referred to are incorporated herein by references.

## EXAMPLE 1

### Construction Of The Vector

A plasmid, pITL was designed to contain minimal exogenous DNA and to be maximally "humanized", with elements of the plasmid except the origin of replication and selection element (supF) being derived from human genetic elements. (Figure 1).

Standard molecular techniques were used for the following manipulations. From piVX a 207 base sequence (SupF) is excised using ECO RI and gel purified. This is ligated into the ECO RI site of the cloning vector pBluescript II KS (Stratagene, La Jolla, CA). The minimal colE1 origin of replication (142) is utilized for plasmid replication and growth within permissive strains of *E. coli*. It was excised from pBR327 (ATCC #37516) using Sau 96 and Bfa I enzymes as a double digest and the 453 base pair fragment gel purified. This fragment is 'polished', that is the overhangs from these digests are filled in with DNA polymerase I (Klenow) large

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fragment. This blunt end, 'polished' fragment is ligated into the Sma I site of pBluescript II KS in which the SupF gene has previously been ligated into the ECORI site. The resultant product of these 2 ligations consists of pBluescript KS with the SupF gene and a second ColI origin of replication, both located within the multiple cloning site.

The splice poly A sequence was synthesized and inserted into the vector pGEM 7f+ between the Hind II and Xho I sites.

The unique directional cloning site or sequence acceptance site is upstream of two separate in frame termination codons, a 3-prime splice (intron), and poly-A sequences (the latter two synthetic sequences based on the human growth hormone gene) (144). These synthetic sequences were directionally inserted into pGEM-7F at the Xho and HindIII sites (Genosys, The Woodlands, TX). This vector is digested with Bam HI and HIND III. Likewise, the product from the minimal colE1, SupF, pBluescriptII KS is digested with these same enzymes. These two resultant purified, double digests are ligated together. The product of this ligation contains pGEM7F+ with the synthetic splice poly A sequence, minimal col E1 origin of replication and SupF gene all with the multiple cloning site.

The stuffer region is isolated from pCDM8 by using the following PCR primers to amplify an approximately 200 base pair portion of the stuffer (the exact PCR product will be 253 bases but, incorporates sequence modifications as detailed below). The upper primer (positive strand or 5') is

CCTCGGTACCTGCCACCATGGCGCGGATTCTTTAT (SEQ. ID. NO. 13) and spans the 2217 to 2252 bases of pCDM8 but, diverges at 2235 to accommodate linking restriction sites in this case the 5' Bgl I cloning site (Figure 2) and a Kpn I site at the upstream, 5' end. This is important later for promoter ligation. The lower primer (negative strand or 3') is CGGCATATGGCCTTAAGGCGCCCGCTTGTTCTGAAGT (SEQ. ID. NO. 14) and spans the 2394 to 2433 bases of pCDM8 but, diverges at 2454 to accommodate linking restriction sites in this case the 3' Bgl I cloning site (see Figure 2) and a Nde I site at the upstream, 5' end. This allows isolation of a fragment without a Bgl I site or any other conflicting sites. This fragment is digested with Nde I and gel purified. The pGEM product from above is digested with Nde I (site engineered into synthetic

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sequence) and Stu I which results in a blunt end on the 5' end. The fragment isolated above is ligated into this digested construct, i.e., Nde I end to Nde I end and blunt to blunt.

The following is the splice poly A signal sequence cloned into the Xho and Hind III sites of pGEM-7Zf reading from Xho site to Hind III site. Bold is intron 3, underline is poly A signal sequence, double underline is cloning site and potential site for internal ribosomal entry sites (IRES), with the remainder from the fifth exon but, following the stop codon:

gCCTTAAgggCCATATggTgAgTggATgCCTTgACCCCaggCggggA  
**TgggggAgACCTgTAgTCagAgCCCCgggCagCACaggCCAATg**  
**CCCgTCCTTCCCCTgCAggATgAgTgAgTgCCTCTCCTggCCC**  
**TggAAgTTgCCACTCCAgTgCCCACCagCCTTgTCCTAATAAAA**  
**TTAAgTTgCATCATTTTgTCTgACTAggTgTCCTCTATAATATTA**  
 T (SEQ. ID. NO. 15)

All triplets in italics are stop codons. After processing of punitive mRNA, excision of intron 3, the sequence will contain duplicate stop codons in two reading frames. The 3rd reading frame can contain a stop codon by mutagenizing the double underlined sequence resulting in deletion of last "g" of the double underlined sequence.

The RANTES promoter construct has a Kpn I site at its 3' end and depending upon the size of the truncation, various restriction sites at the 5' end. From pGL-RANTES the 249 base fragment is excised using Kpn I and Sac I enzymes. The product of the above paragraph is likewise digested with Kpn I and Sac I. The two digests, 249 bases and 1176 bases respectively, are gel purified and ligated together resulting in the intact vector pITL-1.

## EXAMPLE 2

### Construction Of Minimal Promoter Elements For Expression In Skeletal Muscle, Monocytes And Dendritic Cells

The choice of promoter sequence included in the vectors is critical as the promoter must be functional in the mammalian target tissues preferably human tissues and drive the expression of the mRNA at an appropriate level. The human RANTES

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promoter is one promoter useful in the present invention because it is smaller than the CMV or b-actin promoters, is functionally between the two in terms of activity, and is functional in the desired tissues (146). This promoter is highly conserved between human, rat and mouse (146, 147). This promoter has been well characterized and contains myoD like elements functional in skeletal muscle cells along with elements known to be active in cells of the reticuloendothelial system. The minimal promoter elements necessary for expression in lymphocytes has been determined (146, 147). Constructs with a series of 5' truncations of the RANTES promoter upstream of the luciferase reporter are constructed in order to evaluate the minimal promoter elements required for transcriptional activity in cells of interest. Such truncated RANTES promoters include a 249,440 and 990 base pair fragments.

Primary cultures of skeletal muscle cells were purchased (Clonetics, San Diego, CA) and cultured according to manufacturers directions. These cells can be transiently transfected with the control plasmid pGreen Lantern (Gibco, BRL, Gaithersburg, MD) using the cationic lipid mixtures Lipofectin and Lipofectamine (Gibco, BRL, Gaithersburg, MD) with only slight modifications from the manufacturer's suggested protocol. These modifications include trypsinizing cells and replanting 20 minutes before transfection and exposing the cells to the lower concentrations of cationic lipids. The previously described pGL-RANTES promoter constructs, pGL-basic and pGL-control vectors are transfected similarly and luciferase activity is assayed after 72 hours using the Luciferase Assay System (Promega, Madison, WI). To control for transfection efficiency pCMV- $\beta$ Gal (Clontech, San Francisco, CA) is cotransfected with the above plasmids and assayed using Galacto-light Plus (Tropix Inc., Bedford, MA). Luminescence is determined for both assays on an existing LKB scintillation counter.

Monocytes are isolated by elutriation from normal donor leukapheresis packs. The elutriation protocols provides an 90-95% pure monocyte population as evidenced by FACS analysis for CD1-a, CD3, CD14, CD16, CD19, CD45RO, CD56, CD80, HLA-DR and HLA-DQ. The remaining 5% of cells are granulocytes/basophils. FACS analysis of MHC class II and CD25 expression reveals that these cells are not activated after the isolation procedure. Routinely  $5 \times 10^8$  monocytes are isolated from a given leukapheresis pack. The same series of constructs and assay conditions are



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evaluated using these cellular preparation. The transfection procedure are modified for suspended cells, but utilize cationic lipid mixtures are above.

The recently reported ability to isolate dendritic cells (DCs), defined by phenotype and functional assays, from peripheral blood mononuclear cells (148) makes it possible to assay for promoter activity in this cellular population. Following elutriation, monocytes are placed into culture with IL-4 and GM-CSF as reported by Sallusto and Lanzavecchia (148). After 48 hours a representative sample is evaluated by FACS for DC phenotype, i.e., CD3-, CD19-, CD14-, CD1a-c+, CD80+, HLA-DR+, and HLA-DQ+. Upon demonstration of DC phenotype, cationic lipid transient transfections are carried out as above with identical assay procedures followed.

Upon conclusion of these experiments the appropriate promoter fragment is determined based on the activities in the above three cellular preparations. Preferred, is a promoter which drives optimal expression in muscle cells, or antigen presenting cells of the target antigen(s). The promoter fragment is ligated into the pITL vector. This constitutes the base vector. The vector is transfected using the standard heat shock method into frozen competent DH10  $\beta$ /p3 *E. coli* bacteria (Gibco BRL, Gaithersburg, MD), grown under ampicillin and tetracycline selection as previously described. The cloning site of the base vector is the location of all subsequent cloning of reporter gene sequences or target antigen sequences.

The computer generated nucleic acid sequence which approximates the sequence of one base vector, pITL, is as follows:

TGCCATGGCG	CGGATTCTTT	ATCACTGATA	AGTTGGTGGA	CATATTATGT
TTATCAGTGA	TAAAGTGTCA	AGCATGACAA	AGTTGCAGCC	GAATACAGTG
ATCCGTGCCG	GCCCTGGACT	GTTGAACGAG	GTCGGCGTAG	ACGGTCTGAC
GACACGCAAA	CTGGCGGAAC	GGTTGGGGGT	GCAGCAGCCG	GCGCTTTACT
GGCACTTCAG	GAACAAGCGG	GCGCCTTAAG	GGCCATATGG	TGAGTGGATG
CCTTGACCCC	AGGCGGGGAT	GGGGGAGACC	TGTAGTCAGA	GCCCCCGGGC
AGCACAGGCC	AATGCCCCGTC	CTTCCCCTGC	AGTGAGTAGT	GA CTGCCCCG
GTGGGATCCC	TGTGACCCCT	CCCCAGTGCC	TCTCCTGGCC	CTGGAAGTTG
CCACTCCAGT	GCCCACCAGC	CTTGTCCTAA	TAAAATTAAG	TTGCATCATT
TTGTCTGACT	AGGTGTCCTC	TATAATATTA	Taagcttgat	atcgAATTCT
TTCTCAACGT	AACACTTTAC	AGCGGCGCGT	CATTTGATAT	GATGCGCCCC

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GCTTCCCGAT AAGGGAGCAG GCCAGTAAAA GCATTACCCG TGGTGGGGTT  
 CCCGAGCGGC CAAAGGGAGC AGACTCTAAA TCTGCCGTCA TCGACTTCGA  
 AGGTTCGAAT CCTTCCCCCA CCACCATCAC TTTCAAAAGT CCGAAAGAAT  
 Tcctgcagcc cGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA  
 5 GCACCGCCTA CATACCTCGC TCTGCTAATC CTGTTACCAG TGGCTGCTGC  
 CAGTGGCGAT AAGTCGTGTC TTACCGGGTT GGA CTCAAGA CGATAGTTAC  
 CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTTCGTG CACACAGCCC  
 AGCTTGGAGC GAACGACCTA CACCGA ACTG AGATACCTAC AGCGTGAGCA  
 TTGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG  
 10 TAAGCGGCAG GGTCCGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA  
 AACGCCTGGT ATCTTTATAG TCCTGTCGGG TTTCCGCCACC TCTGACTTGA  
 GCGTCGATTT TTGTGATGCT CGTCAGGGGG GCGGAGCCTA TGGAAAAACG  
 CCAGCAACGC GGCCggggga tccggaGAGC TCACTCTAGA TGAGAGAGCA  
 GTGAGGGAGA GACAGAGACT CGAATTTCCG GAGCTATTTT AGTTTTCTTT  
 15 TCCGTTTTGT GCAATTTTAC TTATGATACC GGCCAATGCT TGGTTGCTAT  
 TTTGGAAACT CCCCTTAGGG GATGCCCTC AACTGGCCCT ATAAAGGGCC  
 AGCCTGAGCT GCAGAGGATT CCTGCAGAGG ATCAAGACAG CACGTGGACC  
 TCGCACAGCC TCTCCACAG GTACC 1425 base pairs (SEQ. ID. NO. 16),  
 and variants thereof.

The minimal ColE1 origin of replication comprises base pairs 712 through 1164 of pITL, the SupF sequence comprises base pairs 495 through 701, the minimal RANTES promoter comprises base pairs 1177 through 1425, the stuffer sequence comprises base pairs 1 through 221 and the combined splice and poly A sequences comprises base pairs 222 through 481. Base pairs 482 through 494, 602 through 611, and 1165 through 1176 are extraneous, noncoding sequences derived from plasmids from which the component sequences were excised from (sequences in lower case in pITL sequence).

An enzyme restriction map of pITL is provided in Figure 5.

### EXAMPLE 3

#### Purification Of Vector

The vector, pITL, requires the presence of the p3 helper plasmid for appropriate selection and subsequent isolation. However, the p3 helper plasmid is not part of the therapeutic product. Standard plasmid DNA isolation techniques cannot

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selectively isolate pITL (1.5 - 2.0 kb) from the much larger p3 plasmid (60kb). To avoid administering a contaminating plasmid as part of the polynucleotide vaccine preparation, a separate isolation procedure is established based on anion exchange chromatography. Anion exchange resins have a higher affinity for large DNA molecules than small molecules at any given NaCl concentration. The 30 fold larger p3 plasmid will be retained at a NaCl concentration in the elution gradient which releases the pITL. After standard alkaline/SDS lysis of large volume culture, digestion with RNase A at 100ug/ml, and isopropanol precipitation the resultant pellet is resuspended in TE and applied to the anion exchange column. Small disposable Nucleobond AX columns (Nest Group Inc., Southborough, MA) or MonoQ column (Pharmacia Biotech, Piscataway, NJ) are run on an FPLC system (Pharmacia Biotech, Piscataway, NJ) with a NaCl gradient from 0.01M to 3M. The covalently closed circular (CCC) form of the pITL construct is eluted at less than 1M NaCl while the p3 plasmid is retained. The open circular (OC) form of the pITL plasmid is eluted at a lower NaCl concentration in the gradient and is discarded. Columns are used only once to avoid cross contamination. The resultant retained (CCC) fractions are precipitated with ethanol at -20°C. The pellet is resuspended with sterile PBS and stored aseptically at -20°C until use. Purity of the preparation is assessed by agarose electrophoresis and ethidium bromide staining of undigested and HindIII digested samples. Hind III digests linearize both pITL and the p3 helper plasmid. An ultraviolet spectra across the 240nm to 320 nm range is also obtained on an existing DU 65 UV/Vis spectrophotometer (Beckman, Fullerton, CA). Sample aliquots are retained for microbiological evaluation as needed in the animal experiments. The polynucleotide vector of the present invention is greater than 95% pure, preferably greater than 99% pure, more preferably greater than 99.9% for use in humans.

#### EXAMPLE 4

##### Evaluation of the kinetics of expression of a reporter sequence from the plasmid pITL in an Animal Model

A rat model using Fisher 344 rats is used to evaluate the polynucleotide vaccine vector. The rat model was chosen over mouse to avoid the complication of

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the mouse mammary tumor virus which is present in most mouse mammary tumor lines and which can function as a superantigen causing significant alterations in the immune system. Cohorts of 45 rats, vaccinated with a given dose of DNA are used, for evaluation of toxicity and target sequence expression over a period of 60 days. Three individual members of each cohort are sacrificed and submitted for necropsy on days; 0, 1, 2, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56 in order to evaluate sequence expression and toxicity. Techniques of polynucleotide vaccination have been described previously (102, 132a, 133a, 134a, 107, 119). All animals receive intramuscular injections using standard sterile technique and 27 gauge needles however, in an effort to more accurately mimic the clinical situation the muscle is not surgically exposed. The lateral limb of individual animals is clipped, as needed, and cleansed with 70 % ethanol prior to injections. On day # -1 the left and right gastrocnemius muscle is injected with 200 microlitres of 0.5% bupivacaine-HCL and 0.1% methylparaben in isotonic NaCl ( 0.5% Marcaine, Sanofi Winthrop Pharmaceuticals or 0.5 % Sensorcaine, Astra USA, Inc.). The injection site is identified by a cutaneous tattoo, < 1 mm diameter, with sterile Indian Ink (autoclaved 20 min.) placed at the time of bupivacaine injection. On Day 0 the closed circular DNA preparation in a volume of 200 microlitres of injection grade normal saline is injected into one limb only at the same site as the bupivacaine injection as noted by the tattoo. The opposite limb will be used as a control with an equal volume of sterile injection grade normal saline, without any DNA, administered in the same fashion as above.

The "humanized" nucleic acid sequence encoding a green fluorescent protein sequence portion or variant thereof present in the Green Lantern vector (Gibco BRL, Gaithersburg, MD) is utilized. This vector results in production of a green fluorescent protein in human skeletal muscle cells in vitro and in vivo. This reporter protein has the advantage of not requiring any treatment of tissue to visualize expression and it is stable in both frozen section and formalin fixed paraffin embedded tissues. Using fluorescent microscopy trace expression of this protein is accurately identified using conditions used for evaluation of FITC labeled antibodies. Therefore, this reporter also provides the opportunity to evaluate reticuloendothelial cells by FACS to evaluate possible uptake and expression of the reporter vector. PCR primers to the green lantern protein are used with the appropriate Bgl I 5' extensions to amplify and clone

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the cDNA encoding green fluorescent protein into pITL, in a manner identical to that used to insert tumor target antigen sequences.

The construct containing the green fluorescent protein, pITL-GFP, is sequenced by standard dideoxy sequencing techniques to confirm the fidelity of the cloning process. The nucleic acid sequence for GFP target sequence contained in pITL is as follows:

ATGAGCAAG	GGCGAGGAAC	TGTTCACTGG	CGTGGTCCCA	ATTCTCGTGG
AACTGGATGG	CGATGTGAAT	GGGCACAAAT	TTTCTGTCAG	CGGAGAGGGT
GAAGGTGATG	CCACATACGG	AAAGCTCACC	CTGAAATTCA	TCTGCACCAC
TGGAAAGCTC	CCTGTGCCAT	GGCCAACACT	GGTCACTACC	TTCACCTATG
GCGTGCAGTG	CTTTTCCAGA	TACCCAGACC	ATATGAACGA	GCATGACTTT
TTCAAGAGCG	CCATGCCCGA	GGGCTATGTG	CAGGAGAGAA	CCATCTTTTT
CAAAGATGAC	GGGAACTACA	AGACCCGCGC	TGAAGTCAAG	TTCGAAGGTG
ACACCCTGGT	GAATAGAATC	GAGTTGAAGG	GCATTGACTT	TAAGGAAGAT
GGAAACATTC	TCGGCCACAA	GCTGGAATAC	AACTATAACT	CCCACAATGT
GTACATCATG	GCCGACAAGC	AAAAGAATGG	CATCAAGGTC	AACTTCAAGA
TCAGACACAA	CATTGAGGAT	GGATCCGTGC	AGCTGGCCGA	CCATTATCAA
CAGAACTCTC	CAATCGGCCA	CCGCCCTGTG	CTCCTCCCAG	ACAACAATTA
CCTGTCCACC	CAGTCTGCCC	TGTCTAAAGA	TCCCAACGAA	AAGAGAGACC
ACATGGTCCT	GCTGGAGTTT	GTGACCGCTG	CTGGGATCAC	ACATGGCATG

GACGAGCTGT ACAAGTGAGC (SEQ. ID. NO. 17), and analogs thereof.

The pITL-GFP DNA is prepared as described above, for the vaccination procedure. In one embodiment, the computer generated sequence which approximates the full pITL-GFP sequence comprises:

Tatgagcaagggcgaggaactgttcaactggcggtgggtcccaattctcgtggaactggat  
 ggcgatgtgaatgggcacaaatcttctgtcagcggagaggggtgaaggtgatgccacat  
 acggaaagctcaccctgaaattcatctgcaccactggaaagctccctgtgccatggccaa  
 cactggtcactaccttcacctatggcgtgcagtgtctttccagatacccagaccatat  
 gaagcagcatgactttttcaagagcgccatgcccaggggtatgtgcaggagagaacc  
 atctttttcaaagatgacgggaactacaagaccgcgctgaagtcaagttcgaaggtg  
 acaccctggtgaatagaatcgagttgaagggcattgactttaaggaagatggaaacat  
 tctcgccacaagctggaatacaactataactcccacaatgtgtacatcatggccgac  
 aagcaaaagaatggcatcaaggtcaacttcaagatcagacacaacattgaggatggat

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c c g t g c a g c t g g c c g a c c a t t a t c a a c a g a a c a c t c c a a t c g g c g a c g g c c c t g t g c t  
 c c t c c c a g a c a a c c a t t a c c t g t c c a c c c a g t c t g c c c g t c t a a a g a t c c c a a c g a a a  
 a g a g a g a c c a c a t g g t c c t g c t g g a g t t t g t g a c c g c t g c t g g g a t c a c a c a t g g c a t  
 g g a c g a g c t g t a c a a g t g a g c c a t a t g g t g a g t g g a t g c c t t g a c c c c a g g c g g g g a t  
 5 G G G G G A G A C C T G T A G T C A G A G C C C C G G G C A G C A C A G G C C A A T G C C C G T C C T T C C C C T  
 G C A G T G A G T A G T G A C T G C C C G G G T G G G A T C C C T G T G A C C C C T C C C C A G T G C C T C T C C T  
 G G C C C T G G A A G T T G C C A C T C C A G T G C C C A C C A G C C T T G T C C T A A T A A A A T T A A G T T G C  
 A T C A T T T T G T C T G A C T A G G T G T C C T C T A T A A T A T T A T a a g c t t g a t a t c g A A T T C T T T  
 C T C A A C G T A A C A C T T T A C A G C G G C G C G T C A T T T G A T A T G A T G C G C C C C G C T T C C C G A T  
 10 A A G G G A G C A G G C C A G T A A A A G C A T T A C C C G T G G T G G G G T T C C C G A G C G G C C A A A G G G A  
 G C A G A C T C T A A A T C T G C C G T C A T C G A C T T C G A A G G T T C G A A T C C T T C C C C C A C C A C C A  
 T C A C T T T C A A A A G T C C G A A A G A A T T c c t g c a g c c c G T G T A G C C G T A G T T A G G C C A C C A  
 C T T C A A G A A C T C T G T A G C A C C G C C T A C A T A C C T C G C T C T G C T A A T C C T G T T A C C A G T G  
 G C T G C T G C C A G T G G C G A T A A G T C G T G T C T T A C C G G G T T G G A C T C A A G A C G A T A G T T A C  
 15 C G G A T A A G G C G C A G C G G T C G G G C T G A A C G G G G G T T C G T G C A C A C A G C C C A G C T T G G A  
 G C G A A C G A C C T A C A C C G A A C T G A G A T A C C T A C A G C G T G A G C A T T G A G A A A G C G C C A C G  
 C T T C C C G A A G G G A G A A A G G C G G A C A G G T A T C C G G T A A G C G G C A G G G T C G G A A C A G G A G  
 A G C G C A C G A G G G A G C T T C C A G G G G A A A C G C C T G G T A T C T T T A T A G T C C T G T C G G G T T  
 T C G C C A C C T C T G A C T T G A G C G T C G A T T T T T G T G A T G C T C G T C A G G G G G C G G A G C C T A  
 20 T G G A A A A C G C C A G C A A C G C G G C g g g g g a t c c g g a G A G C T C A C T C T A G A T G A G A G A G  
 C A G T G A G G G A G A G A C A G A G A C T C G A A T T T C C G G A G C T A T T T C A G T T T T C T T T T C C G T T  
 T T G T G C A A T T T C A C T T A T G A T A C C G G C C A A T G C T T G G T T G C T A T T T T G G A A A C T C C C C  
 T T A G G G G A T G C C C C T C A A C T G G C C C T A T A A G G G C C A G C C T G A G C T G C A G A G G A T T C C  
 T G C A G A G G A T C A A G A C A G C A C G T G G A C C T C G C A C A G C C T C T C C C A C A G G T A C C (SEQ  
 25 ID NO. 18) and analogs thereof. The nucleic acid sequence encoding the green  
 fluorescent protein sequence are in lower case and bolded.

Toxicity from polynucleotide vaccination, as reported in the literature (102,  
 132a, 133a, 134a, 135a, 103, 104-131), has been limited to minimal local  
 inflammatory responses at the site of injection. Expression of the target protein, GFP,  
 30 is expected through 60 days. The literature supports extended expression, upwards  
 of 18 months, with other conventional vectors.

Cohort #1 consists of 45 rats each 200 microlitres of normal saline without  
 DNA. Cohort #2 consists of 45 rats each receiving 1 microgram of pITL-GFP closed  
 circular plasmid DNA. Cohort # 3 consists of 45 rats each receiving 10 micrograms  
 35 of pITL-GFP closed circular plasmid DNA. Cohort # 4 consists of 45 rats each

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receiving 100 micrograms of pITL-GFP closed circular plasmid DNA. Cohort # 5 consists of 45 rats each receiving 1 milligram of pITL-GFP closed circular plasmid DNA.

Three animals from each of the cohorts # 1 through 5 are euthanized on day # 0, 1, 2, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56, either by inhaled carbon dioxide or cervical dislocation. Animals are examined at necropsy immediately following euthanasia. The following tissue specimens are submitted for histologic evaluation by H & E stained sections: skeletal muscle and skin from both injection sites, normal skin and skeletal muscle, inguinal lymph node(s), thymus, bone marrow (femur), spleen, gastrointestinal tract with Peyer's patches, heart, lung, liver, kidney, brain/spinal cord, and any other tissues which appear grossly abnormal at the time of necropsy. Tissues from these same organs are prepared and frozen in OCM media for frozen section evaluation of GFP expression by fluorescent microscopy and stored at -70°C for future studies. Blood specimens not to exceed 2 ml are collected by retro-orbital plexus or tail vein phlebotomy no more than weekly for evaluation of hematologic parameters.

If distant or significant local toxicity is detected at any of the DNA dose levels using the pITL-GFP, additional cohorts of 45 animals is treated identically but, administered pITL (without the reporter sequence) at identical dose levels to pITL-GFP and at all subsequent increased dose levels. If no toxicity is seen at any level a single cohort, #5a, 45 additional rats will receive the highest DNA dose level of pITL to confirm the absence of vector derived toxicity.

## EXAMPLE 5

### Evaluation of the impact of tumor presence on the kinetics of polynucleotide vaccine expression, pITL

Previous work by Ochoa and colleagues has suggested that in tumor bearing mice there is a profound immune deficit characterized by T cell receptor and signal transduction defects (149). In light of these observations, the presence of tumor might alter the stability and expression by the polynucleotide vaccine. It is possible that these defects will be manifest in this model and may result in alterations of the

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immune response to the reporter protein. This maybe manifest by increased and persistent expression or a skewing of the immune response with a predominance of antibodies and subsequent ADCC or immune complex deposition. Therefore, these parameters are evaluated in tumor bearing animals. The syngeneic rat mammary tumor line 13762 (ATCC #1666-CRL) which is known to express rat HER2/neu is utilized.

Cohorts # 6 through 10, 30 rats per cohort, involve animals with established 13762 mammary tumors. Rats are injected with  $5 \times 10^5$  cells into the subcutaneous space 14 days prior to receiving polynucleotide vaccination. All rats receive an identical vaccine sequence as that described above. Cohort #6 receive 200 microlitres of normal saline without DNA. Cohort #7 receive 1 microgram of pITL-GFP closed circular plasmid DNA. Cohort #8 receive 10 micrograms of pITL-GFP closed circular plasmid DNA. Cohort #9 receive 100 micrograms of pITL-GFP closed circular plasmid DNA. Cohort #10 receive 1 milligram of pITL-GFP closed circular plasmid DNA.

Three animals from each of the cohorts #6 through 10 are euthanized on Day # 3, 7, 14, 21, 28, 35, 42, 49, and 56 or earlier if animals become moribund due to tumor growth. Animals are necropsied immediately after euthanasia. Tissue specimens are identical to cohorts #1 through 4 with the exception that sections of the tumor nodule is submitted for H&E stained histochemistry and a specimen is frozen as above. Blood specimens not to exceed 2 ml is collected by retro-orbital plexus or tail vein phlebotomy no more than weekly for evaluation of hematologic parameters. Clearly, if tumor growth is either accelerated or sustained this will cause morbidity to the animals.

## EXAMPLE 6

### **Evaluation of immune response and toxicities to anti-tumor vaccination, using constructs with partial sequences of rat HER2/neu, in normal and tumor bearing rats**

The C-erbB-2 or HER-2/neu gene product, originally isolated from neuroblastoma tumor lines, is homologous to the epidermal growth factor receptor (150-154) and expressed in up to 40% of breast carcinomas (26). Since the original



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characterization of this sequence the amplification and overexpression of the protein has been variably associated with prognosis in both ovarian and breast carcinomas (155-165). In murine cell lines, activation of HER-2/neu has been associated with mutations in the transmembrane domain. However, to date most tumor specimens demonstrate gene amplification rather than point mutations (155, 157, 158, 166). HER2/neu amplification occurs in up to one third of breast carcinomas. Monoclonal antibodies recognizing the c-erbB-2 or HER2/neu protein have been shown to be cytostatic in vitro for cell lines overexpressing the c-erbB-2 or HER2/neu protein (167-170). Additionally, CTL lines isolated from patients with ovarian carcinoma have been shown to recognize synthetic peptides corresponding to two regions on the HER2/neu protein (74) despite the fact that c-erbB-2 has been reported to be expressed in the aerodigestive and urologic tract (171). These findings suggest that HER2/neu can be a source of tumor antigens for an anti-tumor immune response.

The choice of HER2/neu as the initial target, tumor-associated, antigen is predicated upon considerations of the animal model and the human clinical trial. A concern of polynucleotide vaccination is the theoretical induction of an autoimmune phenomena to related normal proteins. HER2/neu shares the high degree of homology with other normal cellular proteins, i.e. with other members of the EGFR family (150, 154) as shown in Figure 3. Therefore, a phase 1 clinical trial with this target antigen is most likely to expose this potential toxicity. The human Her2/neu target antigen corresponds with amino acid residues in Figure 3. Additionally, it has been demonstrated that the rat mammary tumor 13762 expresses rat neu. To minimize the potential for autoimmune phenomena a limited partial sequence, including the transmembrane domain (the site of the activating mutation in rat neu), and a segment of the cytoplasmic domain with limited homology to EGFRs is constructed using rtPCR techniques from rat 13762 and the human breast cancer cell line SK-BR-3 (ATCC # HTB-30). These sequences share very limited homology with other EGFR members. Identical sections of the rat and human sequence are utilized. The target amino acid sequence for both human Her2/neu and rat Her2/neu is depicted in Figure 4.

The nucleic acid sequence encoding the partial human Her 2/neu target sequence is ligated at the initiation site of the polynucleotide vector. In one

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embodiment, the nucleic acid sequences encode at least one or a combination of the following amino acid sequences or portion thereof of Her 2/neu:

PDSYMPIWKF	PDEEGACQPC	PINCTHSCVD	LDDKGCPAEQ
RASPLTSIIS	AVVGILLVVV	LGVVFGIL (SEQ. ID NO. 19), or portion	

or mammalian homolog thereof and,

PAPGAGGMVH	HRHRSSSTRS	GGGDLTLGLE	PSEEEAPRSP
LAPSEGAGSD	VFDGDLGMGA	AKGLOSLPTH	DPSPLQRYSE
DPTVPLPSET	DGYVAPLTCS	PQPEYVNQPD	VRPOPPSPRE
GPLPAARPAG	ATLERPKTLS	PGKNGVVKDV	FAFGGAVENP
EYLTPQGTC	PQPEYVNQPD	VRPQPPSPRE	GPLPAARPAG
ATLERPKLSP	GKNGVVKDVF	AFGGAVENPE	YLTPQGGAAP
QPHPPPAFSP	AFDNLYYWDO	DPPERGAPPS	TFKGTPTAEN

PEYLGLDVPV (SEQ. ID NO. 20), or portion or mammalian homolog thereof.

In another embodiment, the nucleic acid sequences encode at least one region corresponding to the transmembrane domain of Her 2/neu comprising the amino acid sequence:

IISAVVGILLVVVLGVVFGILI (SEQ. ID NO. 21), or portion or mammalian homolog thereof.

The respective nucleic acid sequences encoding the target antigen are ligated into pITL. Subsequent clones are sequenced by standard dideoxy sequencing techniques (Sequenase, USB/Amersham, Arlington Heights, IL) to assure fidelity of the insert. Appropriate clones of the vector are transformed into DH10 b/p3, amplified, purified, and characterized as described above. These preparations are used in the following animal experiments.

Cohorts #11 through 15, 20 rats per cohort, receive three repeated injections in the same limb, every three weeks, administered exactly as described above with the exception that the tattoo is placed only once. The polynucleotide vaccine preparation consist of pITL-rHER2/neu, i.e., pITL with a partial rat HER2/neu cDNA sequence. Two weeks after the final injection the rats are challenged with  $5 \times 10^5$  13762 tumor

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cells in no more than 0.5 ml sterile normal saline administered into the subcutaneous space. Cohort #11 receives only sterile saline and no closed circular plasmid DNA per injection. Cohort #12 receives 1 microgram of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #13 receives 10 micrograms of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #14 receives 100 micrograms of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #15 receives 1 milligram of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohorts #16 through #20, 20 rats per cohort, have  $5 \times 10^5$  cells in no more than 0.5 ml sterile normal saline administered into the subcutaneous space 10 to 14 days prior to initiating a polynucleotide vaccine sequence of three injections administered every three weeks so as to have an established tumor not to exceed 1.0 cm in diameter. Cohort #16 receives only sterile saline and no closed circular plasmid DNA per injection. Cohort #17 receives 1 microgram of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #18 receives 10 micrograms of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #19 receives 100 micrograms of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #20 receives 1 milligram of pITL-rHER2/neu closed circular plasmid DNA per injection.

Animals from cohorts #11 through 15 have venous blood samples obtained pre-vaccine initiation, at the time of each vaccination, at the time of tumor challenge, and every three weeks thereafter. These samples are used to assess humoral immune responses by FACS analysis on both 13762 tumor cells and RAT2 cells transiently transfected with the vaccine construct, pITL-rHER2/neu, using cationic lipid techniques as described above for promoter evaluation. A 'sandwich' ELISA is also used to evaluate the development of anti-HER2/neu antibodies, i.e. using test serum as the capture antibody, application of either SKBR3 (HER2/neu + human breast cancer cell line) or 13762 cellular lysates, detection with anti-rat or anti-human HER2/neu antibody ( Ab-4 and Ab-2 or Ab-5 respectively, Oncogene Sciences) and followed by an appropriate anti-isotope enzyme labeled detection antibody and calorimetric determination.

Three individual animals from each cohort are euthanized at the following time points: at the time of tumor challenge, two weeks after tumor challenge, four weeks after tumor challenge, six weeks after tumor challenge, and the remaining animals

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when moribund or no later than one year after tumor challenge. Euthanized animals have spleens harvested for isolation of T lymphocytes, tumor nodule sampling for histochemical examination (both fixed and frozen section), and necropsied with gross abnormalities directing further tissue section submission. Splenic T lymphocytes are isolated used to assay tumor specific CTL precursor frequency using limiting dilution assays (89) and signal transduction defects as previously described for mouse model systems (149, 172).

Animals from cohorts #16 through #20 have venous blood samples obtained pre-tumor inoculation, pre-vaccine initiation, at the time of each vaccination, and every three weeks thereafter. These samples are used to assess humoral immune responses as described above. Three individual animals from each cohort are euthanized at the following time points: at the time of first vaccination (pre treatment), at the time of second vaccination, at the time of the third vaccination, 3 weeks after completion of vaccination, 6 weeks after completion of the vaccination sequence, 12 weeks after completion of the vaccination sequence, and the remaining animals when moribund or no later than one year after completion of the vaccination sequence. Euthanized animals have spleens harvested for isolation of T lymphocytes, tumor nodule sampling for histochemical examination (both fixed and frozen section), and necropsied with gross abnormalities directing further tissue section submission. Splenic T lymphocytes are isolated used to assay tumor specific CTL precursor frequency and signal transduction defects.

#### EXAMPLE 7

##### **Evaluation of toxicity of anti-tumor vaccination with constructs containing partial sequences of human HER2/neu in rats**

Cohorts #21 through 25, 45 rats per cohort, receive three repeated injections in the same limb, every three weeks, administered exactly as described above with the exception that the tattoo is placed only once. The construct used is the base vector with the human partial HER2/neu sequence construct, pITL-hHER2/neu. The nucleic acid sequence of one embodiment of a pITL-hHer2/neu polynucleotide vector vaccine

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comprises:

GCCACCATGGCCcctgacctctectacatgcccctctggaagtttccagatgaggagggcgcatgccagcc  
ttgccccatcaactgcacccactcctgtgtggacctggatgacaagggtgccccgccgagcagagagccagccct  
ctgacgtccatcatctctcggtgggttgacattctgctggctggtcttgggggtggtctttgggatcctcatcaagcg  
acggcagcagaagatcacatgtccagacctgccccgggcgtgggggcatgggtccaccacaggcaccgcagctc  
atctaccaggagtggcggtggggacctgacactagggctggagccctctgaagaggaggccccaggtctccactg  
gcacctccgaaggggctgggtccgatgtattgatgtgacctgggaatgggggcagccaaggggctgcaaagcc  
tccccacatgaccccagccctctacagcgggtacagtgaggaccccacagtacctctgacctctgagactgatggc  
taegtggccccctgacctgcagccccagcctgaatatgtgaaccagccagatgttggccccagccccct  
tcgccccgagaggggccccctctgctgtgccccacctgtggtgccactctggaaaggcccaagactctctccccagg  
gaagaatggggctgctcaaagacgtttttgctttgggggtgccgtggagaacccccgagacttgacaccccaggag  
gagctgccccctagccccacctctcctgcttcagccccagccttcgacaacctctattactgggaccaggacccac  
cagagcgggggggtccaccagcaccttcaaaggacacctacggcagagaaccagagtacctgggtctggac  
gtgccagtgtgaaGCCTTAAGGGCCATATGGTGAGTGGATGCCTTGACCCCAGG  
CGGGGATGGGGGAGACCTGTAGTCAGAGCCCCCGGGCAGCACAGGCCAA  
TGCCCGTCCTTCCCCTGCAGTGAGT AGTGACTGCCCGGGTGGGATCCCTG  
TGACCCCTCCCCAGTGCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGC  
CCACCAGCCTTGTCCTAATAAAATTAAGTTGCATCATTTTTGTCTGACTAGG  
TGTCTCTATAATATTATaagcttg atctcgAATTCTTTCTCAACGTAACTTTA  
CAGCGGCGCGTCATTTGATATGATGCGCCCCGCTTCCCGATAAGGGAGCA  
GGCCAGTAAAAGCATTACCCGTGGTGGGGTTCCCGAGCGGCCAAAGGGA  
GCAGACTCTAAATCTGCCGTCATCGACTTCGAAGGTTTGAATCCTTCCCC  
CACCACCATCACTTTCAAAGTCCGAAAGAATTcctgcagcccGTGTAGCCGTA  
GTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTC  
TGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTT  
ACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTTCGG  
GCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTAC  
ACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCC  
CGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACA  
GGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAG  
TCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTC  
GTCAGGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCgggggatcc

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ggaGAGCTCACTCTAGATGAGAGAGCAGTGAGGGAGAGACAGAGACTCGA  
 ATTTCCGGAGCTATTTTCAGTTTTCTTTTCCGTTTTGTGCAATTTCACTTATG  
 ATACCGGCCAATGCTTGGTTGCTATTTTGGAACTCCCCTTAGGGGATGC  
 CCCTCAACTGGCCCTATAAAGGGCCAGCCTGAGCTGCAGAGGATTCTCTGC  
 AGAGGATCAAGACAGCACGTGGACCTCGCACAGCCTCTCCACAGGTACCT  
 (SEQ ID NO. 22) and analogs thereof. The nucleic acid sequence encoding the  
 human Her 2/neu target sequence are in lower case and bolded.

Three animals from each of the cohorts # 21 through 25 are euthanized on Day  
 # 0, 1, 2, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56, either by inhaled carbon dioxide  
 or cervical dislocation. Animals are examined at necropsy immediately following  
 euthanasia. The following tissue specimens are submitted for histologic evaluation by  
 H & E stained sections: skeletal muscle and skin from both injection sites, normal skin  
 and skeletal muscle, inguinal lymph node(s), thymus, bone marrow (femur), spleen,  
 gastrointestinal tract with Peyer's patches, heart, lung, liver, kidney, brain/spinal  
 cord, and any other tissues which appear grossly abnormal at the time of necropsy.  
 Tissues from these same organs are prepared and frozen in OCM media for frozen  
 section evaluation if indicated and stored at -70°C for future studies. Blood specimens  
 not to exceed 2 ml are collected by retro-orbital plexus or tail vein phlebotomy no  
 more than weekly for evaluation of hematologic parameters.

## EXAMPLE 8

### Phase I Trial

The Phase I study is designed to determine the maximal tolerated dose of a  
 polynucleotide vaccine, evaluate potential toxicities, and immunological effect of the  
 polynucleotide vector vaccine.

### VACCINE PREPARATION

pITL plasmid vector and constructs containing the various target sequences are  
 detailed herein. Individual plasmid constructs have been sequenced to confirm target  
 sequence fidelity. These vectors are transfected using the standard heat shock method  
 into frozen competent DH10  $\beta$ /p3 E. Coli. bacteria (Gibco BRL, Gaithersburg, MD),

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grown under ampicillin and tetracycline selection as previously described. The vector pITL requires the presence of the p3 helper plasmid for appropriate selection and subsequent isolation. However, all current plasmid DNA isolation techniques cannot selectively isolate pITL (1.5 - 2.0 kb) from the much larger p3 plasmid (60kb). To avoid administering a contaminating plasmid as part of the polynucleotide vaccine preparation a separate isolation procedure is disclosed herein based on anion exchange chromatography. Anion exchange resins have a higher affinity for large DNA molecules than small molecules at any given NaCl concentration. The 30 fold larger p3 plasmid is retained at a NaCl concentration in the elution gradient which releases the pITL.

After standard alkaline/SDS lysis of large volume culture, digestion with RNase A at 100ug/ml, and isopropanol precipitation the resultant pellet is resuspended in TE and applied to the anion exchange column. Small disposable Nucleobond AX columns (Nest Group Inc., Southborough, MA) or MonoQ column (Pharmacia Biotech, Piscataway, NJ) run on an FPLC system (Pharmacia Biotech, Piscataway, NJ) are used with a NaCl gradient from 0.01M to 3M. The covalently closed circular (CCC) form of the pITL construct is eluted at less than 1M NaCl while the p3 plasmid is retained and the open circular (OC) form of the pITL plasmid is eluted at a lower NaCl concentration in the gradient and is discarded. Columns are used only once to avoid cross contamination. The resultant retained fractions are precipitated with ethanol at -20°C. The pellet is resuspended with sterile PBS and stored aseptically at -20°C until use. Purity of the preparation is assessed by agarose electrophoresis and ethidium bromide staining of undigested and Hind III digested samples. Hind III digests linearizes both pITL and the 3 helper plasmid. An ultraviolet spectra across the 240nm to 320nm range is also used. Sample aliquots are retained for microbiological evaluation as needed.

### STUDY DESIGN

The patients have histopathological confirmation of the diagnosis of adenocarcinoma of the breast. The specimen submitted to pathology is evaluated for surface expression of HER-2/neu immunohistochemistry. If tumor is available for

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biopsy the immunotherapy laboratory receives 2 cm<sup>3</sup> (minimum of 1 cm<sup>3</sup>) of fresh tumor transported on ice. The sample is used to generate tumor cell and autologous normal breast epithelium or normal fibroblast cell cultures and for mRNA isolation for rtPCR analysis.

5. All patients receive intramuscular (IM) injections (vaccine or control). The vaccine consists of the Her 2/neu polynucleotide vaccine construct as detailed herein at the appropriate dose level per cohort, resuspended in 0.5 ml injection grade, sterile, normal saline and administered in the lateral quadriceps with the patient in the supine position. Cohort #1 receives three initial IM injections of normal saline control (no DNA) prior to receiving three polynucleotide vaccinations.

All intramuscular injections are preceded by IM injection of 0.5 ml. 0.5 % Bupivacaine 24 hours before vaccine administration.

The injection site is marked with a sterile indian ink pinpoint tattoo at the time of the Bupivacaine administration.

#### Cohort #1

Patients receive IM bupivacaine followed by IM injection of sterile saline x 3 at three week intervals. Subsequently, patients receive a dose of 100 microgram of basal plasmid DNA, pITL without the Her 2/neu target sequence insert, per vaccination administered in the contralateral lower extremity at three week intervals x 3.

The first three patients have a muscle biopsy of the sterile saline injection site at week 12. The last three patients have a muscle biopsy of the basal plasmid injection site three weeks after the last vaccination, i.e. week 21.

In this cohort alone, if no toxicity is identified, the patients are vaccinated with the pITL-Her 2/neu construct, in the lower extremity originally injected with bupivacaine and saline. The dose of the pITL-Her 2/neu construct is the highest dose at which two patients have been vaccinated at least twice without observed toxicity greater than grade 2.



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## Cohort #2

The initial vaccination consists of 1 microgram of the reporter plasmid, pITL with the PCR amplified GFP sequence. Patients receive a dose of 1 micrograms of pITL-Her 2/neu DNA per vaccination administered in the contralateral lower extremity at three week intervals x 3. Groups of 2 patients have a muscle biopsy of the reporter plasmid vaccination site at weeks 3, 6 and 9 respectively.

## Cohort #3

The initial vaccination consists of 10 micrograms of the reporter plasmid, pITL with the PCR amplified GFP sequence. A dose of 25 micrograms pITL-Her 2/neu DNA per vaccination is administered in the contralateral lower extremity at three week intervals x 3. Groups of 2 patients have a muscle biopsy of the reporter plasmid vaccination site at weeks 3, 6, and 9 respectively.

## Cohort #4

The initial vaccination consists of 25 micrograms of the reporter plasmid, pITL with the PCR amplified GFP sequence. A dose of 100 micrograms pITL-Her 2/neu DNA per vaccination is administered in the contralateral lower extremity at three week intervals x 3. Groups of 2 patients have a muscle biopsy of the reporter plasmid vaccination site at weeks 3, 6, and 9 respectively.

## Cohort #5

The initial vaccination consists of 100 micrograms of the reporter plasmid, pITL with the PCR amplified GFP sequence. A dose of 1000 micrograms pITL-Her 2/neu DNA per vaccination is administered in the contralateral lower extremity at three week intervals x 3. Groups of 2 patients have a muscle biopsy of the reporter plasmid vaccination site at weeks 3, 6, and 9 respectively.

All patients are evaluated for anti-tumor responses at the conclusion of the vaccination sequence, every two months x 3, then every three months. Patients free from progression at 6 months are eligible for repeated vaccination sequences identical to that initially administered minus the initial vaccination with the reporter plasmid.

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## EVALUATION

Medical history, T lymphocyte function, fluorescent cell sorting, antigen specific responses are performed as detailed in Example 9 below.

## DOSE ADJUSTMENT AND MODIFICATIONS

The maximally tolerated dose (MTD) of total DNA per vaccination is defined as that dose at which 0 or 1/6 patients experience polynucleotide vaccine related dose limiting toxicity (DLT) with the next higher dose level provoking DLT in any 2 patients from that cohort.

If dose limiting toxicity is observed in the first two patients of any cohort, the dose is reduced by 50% and the equivalent of a full cohort enrolled at this reduced dose unless dose limiting toxicity again encountered at this new dose.

### **EXAMPLE 9**

#### **Phase I/II Evaluation Of Polynucleotide Vaccination In Advanced Breast Cancer**

##### **Polynucleotide Vaccine Preparation**

The patients undergo an excisional biopsy with histopathological confirmation of the diagnosis of adenocarcinoma of the breast. The specimen submitted to pathology is also used for evaluation of surface expression of HER-2/neu and MUC-1 by immunohistochemistry. The immunotherapy laboratory receives 2 cm<sup>3</sup> (minimum of 1 cm<sup>3</sup>) of fresh tumor transported on ice. This specimen is divided with 50% of the sample dispersed with collagenase and the resultant cellular preparation cryopreserved for use as described below. 10-15% of the sample is used to generate tumor cell and autologous normal breast epithelium or normal fibroblast cell cultures. The remainder is used for mRNA isolation and vaccine preparation with an immediately adjacent slice/section held for histologic confirmation and correlation. The sample is homogenized in TriZol solution (Gibco BRL) for isolation of total RNA. Messenger RNA is isolated with oligo dT agarose (Pharmacia) and subjected

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to rtPCR with first strand reverse transcription initiated from oligo d(T)12N using StrataScript Rnase H- Reverse Transcriptase (Stratagene) and the high fidelity pfu (Stratagene) thermostable polymerase for the PCR reaction. (Chapter 15 in: Current Protocols in Molecular Biology, Vol. 2; Ausubel, F.M. et al (eds) John Wiley & Sons, Inc. 1995).

Specific PCR primers for the individual transcripts of interest are used in individual PCR reactions using an aliquot of the cDNA from the reverse transcription of the mRNA. An embodiment of rtPCR primers for the target sequence p53 is as follows:

p 53 rtPCR primer 1: g tctgccacca tggcctactc ccctgc (SEQ. ID. NO. 23) and variants thereof;

p 53 rtPCR primer 2: ttc ttggtgacc tacctcttcg gaattgccga gtc (SEQ. ID. NO. 24) and variants thereof.

The location of the primer extension on the P53 cDNA using primer 1 and primer 2 may be depicted as follows:

atggaggagccgcagtcagatcctagcgtcagccccctctgagtcaggaaacattttca  
gacctatgaaactacttctgaaaacaacgttctgtcccccttgcctcccaagcaatg  
gatgatttgatgctgtccccggacgatattgaacaatggttctactgaagaccaggtcca  
gatgaagctcccagaatgccagagcgtgctccccgcgtggccccctgcaccagcagctcct  
acaccggcgccccctgcaccagccccctctggccccctgtcattctgttcccttccag  
aaaacctaccagggcagctacggttccgtctgggcttctgtcattctgggacagccaa  
**gtctgccacca tggcctactc ccctgc →**

(primer 1)

gtctgtgacttgacgtactccccctgcccccaacaagatgttttccaactggccaagacc  
tgcctgtgcagctgtgggttgattccacacccccgccggcaccgcgtccgcgccatg  
gccatctacaagcagtcacagcacatgacggaggtgtgagcgctgccccaccatgag  
cgctgctcagatagcgatggctgtgccccctctcagcgtcttatccgagtggaaaggaaat  
ttgcgtgtggagtatttgatgacagaaacacttttcgacatagtgtggtggtgccctat  
gagccgcctgaggttgctctgactgtaccaccatccactacaactacatgtgtaacagt  
tctgcattggggcgcatgaaccggaggccatcctcaccatcatcacactggaagactcc  
agtggtaactactgggacggaacagctttgaggtgcgtgtttgtgcctgtcctgggaga  
gaccggcgccacagaggagaagaatctccgaagaaaggggagcctcaccacgagctgccc  
ccaggggagcactaagcgagcactgcccacaacaccagctcctctccccagccaaag  
aagaaccactggatggagaatatttcaccttcagatccgtgggcgtgagcgcttcgagatg

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-ttcttttggtgacc taccttctcg gaattgccga gtc

(primer 2)

ttccgagagc tgaatgaggc cttggaactc aaggatgcc aggcgtgggaa ggagccaggg

gggagcaggg ctactccag ccacctgaag tccaaaaagg gtcagtctac ctcccgccat

aaaaaactca tgttcaagac agaagggcct gactcagac (SEQ. ID. NO. 25)

Primers from cDNA of ras, RB1, BRCA-1, and TRE17 which incorporate the underlined mutated sequences as shown above for the p53 primers allow the target sequences to be ligated into the polynucleotide vector.

The amplified fragments is digested with the restriction endonuclease Bgl I and ligated, using T4 DNA ligase, into a similarly digested pITL vector which has been dephosphorylated with calf alkaline phosphatase. Ligation products is electroporated into DH10/p3 bacteria and selected on LB ampicillin and tetracycline plates. Ten to twenty individual colonies from each ligation/transfection are randomly selected and subjected to PCR-based sequencing. The nucleic acid sequences from clones positive for mutations are used in preparing a polynucleotide vector vaccine. In one embodiment, a mutation from germline is considered a true mutation and selected for use if present in 20% or greater of the colonies evaluated. If two or more mutations meet the above criteria all clones considered valid is used in the polynucleotide vaccine preparation. Individual clones are isolated from the strain DH10/p3 of *E. coli* using standard molecular biological techniques.

The plasmid of interest is separated from the p3 helper plasmid, required for selection, via ion exchange chromatography using the DEAE 4000-7 resin of the Nest Group Inc. An aliquot of each component of the vaccine preparation is analyzed by agarose gel electrophoresis and ethidium bromide visualization to assure purity of DNA. An aliquot of these large scale preparations is evaluated for nucleotide sequence to confirm the inserted target sequence. Individual aliquots of plasmid preparations is stored at -20°C as an ethanol precipitate (under 70% ethanol in water). Prior to use, these precipitates are resuspended at a concentration of 1 milligram per milliliter (mg/ml) in sterile, injection grade, normal saline. Appropriate volumes are diluted as required to match the individual and total dose parameters of each cohort.

All patients receive intramuscular (IM) injections (vaccine or control). The vaccine consist of individual polynucleotide vaccine construct mixtures at the

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appropriate dose level per cohort, resuspended in 0.5 ml injection grade, sterile, normal saline and administered in the lateral quadriceps with the patient in the supine position. Cohort #1 receives three initial IM injections of normal saline control (no DNA) prior to receiving three polynucleotide vaccinations.

5 All intramuscular injections are preceded by IM injection of 0.5 ml. 0.5 % Bupivacaine 24 hours before vaccine administration. The injection site are marked with a sterile indian ink pinpoint tatoo at the time of the bupivacaine HCl administration.

10 Cohort #1

Patients receive IM bupivacaine HCl followed by IM injection of sterile saline x 3 at three week intervals.

15 Subsequently, patients receive full compliment polynucleotide vaccination, a total dose of 1 microgram of DNA per construct per vaccination with a total dose of DNA not to exceed 5 micrograms per injection, is administered in the lower extremity at three week intervals x 3.

Cohort #2

20 The initial vaccination consists of 10 micrograms of the control plasmid, pITL without any PCR amplified sequences.

Full compliment polynucleotide vaccination, a total dose of 10 micrograms of DNA per construct per vaccination with a total dose of DNA not to exceed 50 micrograms per injection, is administered in the contralateral lower extremity at three week intervals x 3.

25 The first three patients have a muscle biopsy of the control plasmid injection site at week three. The second three patients have a muscle biopsy of the control plasmid injection site at week 6. The final four patients have a muscle biopsy of the control plasmid injection site at week 9.

30 Cohort #3

The initial vaccination consists of 10 micrograms of the reporter plasmid, pITL with the PCR amplified GFP sequence.

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Full compliment polynucleotide vaccination, a total dose of 25 micrograms of DNA per construct per vaccination with a total dose of DNA not to exceed 125 micrograms per injection, will be administered in the contralateral lower extremity at three week intervals x 3.

5 The first three patients have a muscle biopsy of the control plasmid injection site at week three. The second three patients have a muscle biopsy of the control plasmid injection site at week 6. The final four patients have a muscle biopsy of the control plasmid injection site at week 9.

#### 10 Cohort #4

Full compliment polynucleotide vaccination, at a total dose of 100 micrograms of DNA per construct per vaccination with the total dose of DNA per injection not to exceed 500 micrograms, is administered in one lower extremity at three week intervals x 4.

15 All patients are evaluated for anti-tumor responses at the conclusion of the vaccination sequence, every two months x 3, then every three months. Patients free from progression at 6 months are eligible for repeated vaccination sequences identical to that initially administered.

20 Freshly excised tumor is used not only to generate individual patient polynucleotide vaccine preparation but, also for the establishment of tumor cell lines using previously described techniques (173-175) and cryopreserved to evaluate tumor specific CTLp. Normal skin fibroblasts or normal autologous breast epithelium derived from the excisional biopsy is used in transfection studies for analysis of immune responses to individual vaccine components. In the absence of established  
25 cultures of autologous fibroblasts or breast epithelium, autologous PBMC is used in the same fashion although, transfection procedures require non-specific activation and will be adjusted accordingly.

30 Patients of cohort #1 have leukapheresis performed pre-treatment, after the first three injections (just prior to first polynucleotide vaccination), at the first post treatment follow-up visit, and at the 6 month time point. Patients of cohorts #2, #3 and #4 have leukapheresis performed pre-treatment, at the first post treatment follow-up visit, and at the 6 month time point. Manual leukapheresis of not less than

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100 cc is performed for the purpose of evaluation of tumor-specific CTLp and peripheral blood mononuclear cell phenotype at one year after initiating treatment in all surviving patients.

#### T Lymphocyte Function

The parameters monitored are as follows:

1. z chain, lck, and fyn proteins by western blot,
2. nuclear binding factors for  $\gamma$ IFN and NFkB probe sequences,
3. Lymphokine production (IL-2, IL-4, IL-6, IL-10, and  $\gamma$ IFN), and
4. Tumor specific CTL precursors (evaluated by limiting dilution assay)

#### Fluorescent Activated Cell Sorting (FACS)

The phenotype of peripheral blood mononuclear cells is evaluated by flow cytometry from pretreatment, pre-third vaccination, first post-vaccination follow-up visit, and 6 month samples. The cell markers to be determined are; CD3, CD4, CD8, CD16, CD25, CD56, TCR  $\alpha/\beta$ , TCR  $\gamma/\delta$ , CD45 RO, CD45 RA, CD20.

#### Antigen Specific Responses

Non-malignant autologous cell cultures, either fibroblast or breast epithelium depending upon materials obtained from the excisional biopsy, is transiently transfected with a mixture of pITL-GFP and an individual pITL-target sequence construct. Either lipofectin or other established transfection procedures (176) are used. A control transfection of pITL-GFP is utilized for background, non-target sequence, immune responses while the mixture assures that transfection efficiency can be controlled for.

Standard proliferation assays using  $^3$ H-thymidine incorporation (177) are performed using PBMC from individual time points. PBMC is placed into culture with irradiated (10-20cGy) transfected cells as stimulators and after 48,  $^3$ H-thymidine is added for a 16 hour pulse. Cells is harvested and counted in the standard fashion.

The frequency of CTL precursors (CTLp) for any individual component is expected to be on the order of  $1/10^6$  (178) and therefore to assess changes in the CTL compartment isolated T cells from PBMC preparations are non-specifically

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stimulated with OKT3 and provide IL-2 for one week. At the conclusion of this period the bulk culture are divided and assayed for cytotoxicity on the individually transfected autologous cells noted above along with K562 as a control for NK activity.

Tumor specific CTL precursors are analyzed using irradiated (10-20cGy) autologous tumor cells as stimulators in limiting dilution cultures of PBMC from individual time points, using a previously established technique (178). Standard cytotoxicity assays again using autologous tumor cells and K562 as target cells, are scored and tumor specific CTLp frequency calculated.

Muscle biopsies is performed as out patient minor surgery procedures. The tissue samples is multiply divided cross sectionally and separated in an alternating fashion into two groups; the first for standard formalin fixation with H&E histologic examination along with fluorescent microscopy on unstained sections for detection of GFP, the second for frozen section examination and use for immunohistochemical staining. Standard post biopsy care with adequate analgesia and wound care is administered.

#### CRITERIA FOR RESPONSE

##### Complete Response

Disappearance of all clinical and laboratory signs and symptoms of active disease for a minimum period of four weeks. Persistently abnormal bone scans, if stable for a period of not less than 8 weeks is allowed.

##### Partial Response

A minimum of 50% reduction in the sum of the bidimensional product of measurable lesions persisting for a minimum period of four weeks. Although every lesion need not demonstrate a full 50% reduction in bidimensional product, no lesion may grow nor can there be new lesions demonstrated in this category of response.

##### Stable Disease

No change or decrease in the sum of the bidimensional product less than 50% persisting for a minimum period of four weeks. No lesion may grow and no new lesions can appear in this category of response.



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Progressive Disease

25 % increase in the sum of the bidimensional product or the appearance of new lesions.

## TOXICITY

Toxicities is graded according to the NCI Common Toxicity Criteria.

## DOSE ADJUSTMENT AND MODIFICATIONS

The maximally tolerated dose (MTD) of total DNA per vaccination is defined as that dose at which 0 or 1/6 patients experience polynucleotide vaccine related dose limiting toxicity (DLT) with the next higher dose level provoking DLT in any 2 patients from that cohort.

If dose limiting toxicity is observed in the first two patients of any cohort, the dose is reduced by 50% and the equivalent of a full cohort is enrolled at this reduced dose unless dose limiting toxicity is again encountered at this new dose.

If an individual component at a given dose is suspected to be responsible for undue toxicity due to its association with toxicities in separate patients, it is decreased to the previously tolerated dose or dropped from the vaccine preparation if no tolerated dose is previously documented.

If the patient has sufficient potential tumor antigenic templates identified so as to exceed the total dose limitations, if all constructs were to be used, the constructs included in the vaccine preparation is prioritized as follows: 1.) constructs expressing mutated sequences isolated from the patient's tumor, 2.) sequences for Int-2 and Hst and 3.) muc-1 and c-erbB-2/HER2/neu.

## REMOVAL OF PATIENTS FROM PROTOCOL

- A. All three of the following, which preclude polynucleotide vaccine production:
- 1.) No detectable genetic deviation from germline in p53, H or K ras, RB, BRCA-1, TRE2/TRE17,
  - 2.) Absence of overexpression of c-erbB-2/HER 2/neu
  - 3.) No expression of MUC-1
- B. Progressive disease

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- C. Unacceptable toxicity
- D. Intercurrent Illness which prevents further administration of the treatment
- E. Decision of the patient to withdraw from the study
- F. General or specific changes in the patient's condition which render the patient unacceptable for further treatment in the judgement of the investigator.

#### EXAMPLE 10

##### Construction of pITL-1

In this embodiment, the polynucleotide vector comprises a 440 base pair region from the RANTES promoter in combination with a 635 base pair region of colE1 origin of replication. The polynucleotide vector was constructed as follows:

(A) Splice & poly A sequence was synthesized and inserted into the vector pGEM between the Hind III and Xho I site forming pGEM ELN.

(B) The stuffer region was isolated from pCDM8 by PCR amplification of an approximately 200 base pair portion and blunt end ligated into Xho I site of pGEM ELN forming pGEM ELN/Stuffer.

(C) A 440 base pair region of the RANTES promoter was excised from pGL RANTES using Xho I and Nco I. This fragment is klenow filled (polished) and blunt end ligated into pGEM ELN at the Hind III and Nsi site forming pGEM ELN/RANTES.

(D) The 440 base pair region of the RANTES promoter was next excised from pGEM ELN/RANTES using Nsi and Hind III and Inserted into pGEM ELN/Stuffer at its analogous site forming pGEM/ELN/Stuffer/RANTES.

(E) A 635 base pair region of the colE1 origin of replication was excised from pBR327 using Bst YI and Ava I. This fragment was polished and blunt end ligated into pBluescript/Sup F forming pBluescript/Sup F/colE1.

(F) Sup F was excised from  $\pi$  VX using Eco RI and ligated into the Eco RI site of pBluescript forming pBluescript/Sup F.

(G) The Sup F and colE1 region was removed from pBluescript/ Sup F/colE1 using Hind III and Bam HI and inserted into pGEM/ ELN/Stuffer/RANTES at its analogous site forming pGEM/pITL-1.

(H) pITL-1 is separated from pGEM by excising with Kpn I. pITL-1 is then religated.

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The resulting vector is designated herein as pITL-1. The pITL-1 vector contains the fragment of the RANTES promoter that corresponds with the region in genomic RANTES promoter from about NCO site through the KpnI site. The plasmid polynucleotide vector in *Escherichia Coli* strain DH 10 $\beta$ /P3 was deposited under terms of the Budapest treaty with the American Type Culture Collection, 12301 Parklawn Drive, Rockville MD 20852 USA on April 9, 1997 under ATCC Designation 98400.

The computer generated sequence which approximates the sequence of pITL-1 comprises the sequence:

GgtacctgccaccaTGGCGCGGATTCTTTATCACTGATAAGTTGGTGGACATATT  
ATGTTTATCAGTGATAAAGTGTCAAGCATGACAAAGTTGCAGCCGAATAC  
AGTGATCCGTGCCGGCCCTGGACTGTTGAACGAGGTCGGCGTAGACGGTC  
TGACGACACGCAAACCTGGCGGAACGGTTGGGGGTGCAGCAGCCGGCGCT  
TTACTGGCACTTCAGGAACAAGCGGGCGCcttaaggccaTATGGTGAGTGGAT  
GCCTTGACCCAGGCGGGGATGGGGGAGACCTGTAGTCAGAGCCCCCGG  
GCAGCACAGGCCAATGCCCGTCCTTCCCCTGCAGGATgAGTagtgagtgcctcct  
gGCCCTGGaagttgccactccagtGCCaccagccttgctctaataaaattaagttgcatcatttgcctgactaggtg  
cctctataatattataagcttgatcGAATTCTTTCGGACTTTTGAAAGTGATGGTGGTGG  
GGGAAGGATTCGAACCTTCGAAGTCGATGACGGCAGATTTAGAGTCTGCT  
CCCTTTGGCCGCTCGGGAACCCACCACGGGTAATGCTTTTACTGGCCTG  
CTCCCTTATCGGGAAGCGGGGCGCATCATATCAAATGACGCGCCGCTGTA  
AAGTGTTACGTTGAGAAAGAATTCctgcagcccGCCGCGTTGCTGGCGTTTTTC  
CATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCA  
GAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTG  
GAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATAC  
CTGTCCGCCTTTCTCCCTTCGGGAAGCGTGCGCTTTCTCAATGCTCACGC  
TGTAGGTATCTCAGTTCGGTGTAAGTTCGCTCCAAGCTGGGCTGTGTG  
CACGAACCCCCCGTTACGCCCAGCGCTGCGCCTTATCCGGTAACATATCG  
TCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCA  
CTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTC  
TTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTAT  
CTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTT  
GATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAG  
CAGCAGATTACGCGCAGAAAAAAGGATCTgggggatccggagagctcccaacgcgttg  
atgcatggatgagggaaaggaggaagatctgtaataagcaggaacttgaagactcagtgactcagtgaataaa  
gactcagtgaactctgactctgcttaactgccactcctgtgtccaagaaagcggtcctgctctgaggaggaccctt  
ccctggaaggtaaaactaaggatgtcagcagagaaattttccaccattggtgcttggtcaagaggaaactgatGAGC  
TCACTCTAGATGAGAGAGCAGTGAGGGAGAGACAGAGACTCGAATTTCC  
GGAGCTATTTTCAGTTTTCTTTTCCGTTTTGTGCAATTTCACTTATGATACC  
GGCCAATGCTTGGTTGCTATTTTGGAAACTCCCCTTAGGGGATGCCCTC  
AACTGGCCCTATAAAGGGCCAGCCTGAGCTGCAGAGGATTCCTGCAGAGG  
ATCAAGACAGCACGTGGACCTCGCACAGCCTCTCCACA (SEQ ID NO. 28)

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It can be appreciated by those in the art that the actual nucleic acid sequence of the ATCC deposited plasmid may vary slightly from the computer generated sequence due to slight variations in the ligation sites of the various components that comprise the vector. Such variations will not affect the functioning of the vector. The actual sequence of the deposited vector including the ligation sites may be performed using routine sequencing methods as are known in the art.

A smaller polynucleotide vector was constructed, differing from pITL-1 by about 200 base pairs, was constructed as described above with the exception that a base pair region of approximately 220 base pairs of the RANTES promoter was used. The resulting vector is referred to herein as pITL-A. This smaller vector in *E. coli* strain DH-10 $\beta$ /P3 was deposited under terms of the Budapest treaty with the ATCC on April 9, 1997 under ATCC designation 98401. The computer generated sequence which approximates the sequence of pITL-A comprises the sequence:

GgtacctgccaccaTGGCGCGGATTCTTTATCACTGATAAGTTGGTGGACATATT  
ATGTTTATCAGTGATAAAGTGTCAAGCATGACAAAGTTGCAGCCGAATAC  
AGTGATCCGTGCCGGCCCTGGACTGTTGAACGAGGTCGGCGTAGACGGTC  
TGACGACACGCAAACCTGGCGGAA CGGTTGGGGGTGCAGCAGCCGGCGCT  
TACTGGCACTTCAGGAACAAGCGGGCGCcttaaggccaTATGGTGAGTGGAT  
GCCTTGACCCCAGGCGGGGATGGGGGAGACCTGTAGTCAGAGCCCCCGG  
GCAGCACAGGCCAATGCCCGTCCTTCCCCTGCAGGATgAGTgtagtgcctctcct  
gGCCcTGaagttgccactccagtGCCaccagcctgtcctaataaaattaagttgcatcatttgtctgactaggtg  
cctctataatattataagcttgatacGAATTCTTTTCGGACTTTTGAAAGTGATGGTGGTGG  
GGGAAGGATTTCGAACCTTCGAAGTCGATGACGGCAGATTTAGAGTCTGCT  
CCCTTTGGCCGCTCGGGAACCCACCACGGGTAATGCTTTTACTGGCCTG  
CTCCCTTATCGGGAAGCGGGGCGCATCATATCAAATGACGCGCCGCTGTA  
AAGTGTTACGTTGAGAAAGAATTcctgcagcccGCCGCGTTGCTGGCGTTTTTC  
CATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCA  
GAGGTGGCGAAACCCGACAGGACTATAAAGATAACCAGGCGTTTCCCCCTG  
GAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATAC  
CTGTCCGCTTTCTCCCTTCGGGAAGCGTGCGCTTTCTCAATGCTCACGC  
TGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTG  
CACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACATCG  
TCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCA  
CTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTC  
TTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTAT  
CTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTT  
GATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAG  
CAGCAGATTACGCGCAGAAAAAAGGATCTgggggatccggagagctcACTCTAGA  
TGAGAGAGCAGTGAGGGAGAGACAGAGACTCGAATTTCCGGAGCTATTTT  
AGTTTTCTTTTCCGTTTTGTGCA ATTTCACTTATGATAACCGGCCAATGCTT

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GGTTGCTATTTTGGAACTCCCCTTAGGGGATGCCCCCTCAACTGGCCCTAT  
 AAAGGGCCAGCCTGAGCTGCAGAGGATTCCTGCAGAGGATCAAGACAGC  
 ACGTGGACCTCGCACAGCCTCTCCCACA (SEQ ID NO. 27)

# EXAMPLE 11

## pITL-1-GFP

The target reporter gene encoding green fluorescent protein was inserted into the above pITL-1 polynucleotide vector forming the vector, pITL-1GFP. The computer generated sequence which approximates the sequence of pITL-1 GFP comprises:

GgtacctgccaccaTGGCGAAGGGCGAGGAACTGTTCACTGGCGTGGTCCCAATT  
 CTCGTGGAAGCTGGATGGCGATGTGAATGGGCACAAATTTTCTGTCAGCGG  
 AGAGGGTGAAGGTGATGCCACATACGGAAGCTCACCTGAAATTCATCT  
 GCACCACTGGAAAGCTCCCTGTGCCATGGCCAACACTGGTCACTACCTTC  
 ACCTATGGCGTGCAGTGCTTTTCCAGATACCCAGACCATATGAAGCAGCA  
 TGACTTTTTCAAGAGCGCCATGCCCCGAGGGCTATGTGCAGGAGAGAACCA  
 TCTTTTTCAAAGATGACGGGAAGCTACAAGACCCGCGCTGAAGTCAAGTTC  
 GAAGGTGACACCCTGGTGAATAGAATCGAGTTGAAGGGCATTGACTTTAA  
 GGAAGATGGAAACATTCTCGGCCACAAGCTGGAATACAAGTATAACTCCC  
 ACAATGTGTACATCATGGCCGACAAGCAAAAGAATGGCATCAAGGTCAA  
 CTTCAAGATCAGACACAACATTGAGGATGGATCCGTGCAGCTGGCCGACC  
 ATTATCAACAGAACACTCCAATCGGCGACGGCCCTGTGCTCCTCCCAGAC  
 AACCATTACCTGTCCACCCAGTCTGCCCTGTCTAAAGATCCCAACGAAAA  
 GAGAGACCACATGGTCCTGCTGGAGTTTGTGACCGCTGCTGGGATCACAC  
 ATGGCATGGACGAGCTGTACAAGTGAGCGCcttaaggccaTATGGTGAGTGGA  
 TGCTTGACCCAGGCGGGGATGGGGGAGACCTGTAGTCAGAGCCCCCGG  
 GCAGCACAGGCCAATGCCCGTCCCTTCCCCTGCAGGATgAGTAgtagtgccctcct  
 gGCCCTGgaagtgcactccagtGCCaccagcctgtcctaataaaattaagtgcatcatttgtctgactaggtg  
 cctctataatattataagcttgatcGAATTCTTTTCGGACTTTTGAAAGTGATGGTGGTGG  
 GGGAAAGGATTCGAACCTTCGAAGTCGATGACGGCAGATTTAGAGTCTGCT  
 CCCTTTGGCCGCTCGGGAACCCCAACACGGGTAATGCTTTTACTGGCCTG  
 CTCCCTTATCGGGAAGCGGGGCGCATCATATCAAATGACGCGCCGCTGTA  
 AAGTGTTACGTTGAGAAAGAATTCTctgcagcccGCCGCGTTGCTGGCGTTTTTC  
 CATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCA  
 GAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTG  
 GAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATAC  
 CTGTCCGCTTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGC  
 TGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTG  
 CACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAAGTATCG  
 TCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCA  
 CTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTC  
 TTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTAT

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CTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTT  
GATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAG  
CAGCAGATTACGCGCAGAAAAAAGGATCTgggggatccggagagctcccaacgcgttg  
atgcatggatgagggaaaggaggaatctgtaataagaagcaggaacttgaagactcagtgactcagtgaataaaa  
gactcagtgacttctgactctgtcctaactgccactcctgtgttccaagaaagcgcttctgctctgaggaggaccctt  
ccctggaaggtaaaactaaggatgtcagcagagaaatcttccaccattgggtgcttgggtcaaagaggaaactgatGAGC  
TCACTCTAGATGAGAGAGCAGTGAGGGAGAGACAGAGACTCGAATTTCC  
GGAGCTATTTTCAGTTTTCTTTTCCGTTTTGTGCAATTTCACTTATGATACC  
GGCCAATGCTTGGTTGCTATTTTGGAAACTCCCCTTAGGGGATGCCCCCTC  
AACTGGCCCTATAAAGGGCCAGCCTGAGCTGCAGAGGATTCCTGCAGAGG  
ATCAAGACAGCACGTGGACCTCGCACAGCCTCTCCCACA (SEQ ID NO. 29)

The pITL-1-GFP vector was tested for functional expression in primary human skeletal muscle cells. The vector pITL-GFP expressed the reporter protein, GFP in the human skeletal muscle cells in vitro.

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SEQUENCE LISTING

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Nelson, Peter J.
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- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 MB
  - (B) COMPUTER: IBM PC COMPATIBLE
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: WORDPERFECT 5.1
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: PCT/US97/14306
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 453 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:1:

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GAAACCCGAC	AGGACTATAA	AGATACCAGG	CGTTTCCCCC	120
TGGAAGCTCC	CTCGTGCGCT	CTCCTGTTCC	GACCCTGCCG	160
CTTACCGGAT	ACCTCTCCGC	CTTTCTCCCT	TCGGGAAGCG	200
TGGCGCTTTC	TCAATGCTCA	CGCTGTAGGT	ATCTCAGTTC	240
GGTGTAGGTC	GTTCGCTCCA	AGCTGGGCTG	TGTGCACGAA	280
CCCCCGTTTC	AGCCCGACCG	CTGCGCCTTA	TCCGGTAACT	320
ATCGTCTTGA	GTTCAACCCG	GTAAGACACG	ACTTATCGCC	360
ACTGGCAGCA	GCCACTGGTA	ACAGGATTAG	CAGAGCGAGG	400
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 453 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:2:

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GGCTGCTGCC	AGTGGCGATA	AGTCGTGTCT	TACCGGGTTG	120
GACTCAAGAC	GATAGTTACC	GGATAAGGCG	CAGCGGTCGG	160
GCTGAACGGG	GGGTTCGTGC	ACACAGCCCA	GCTTGGAGCG	200
AACGACCTAC	ACCGAACTGA	GATACCTACA	CCGTGAGCAT	240
TGAGAAAGCG	CCACGCTTCC	CGAAGGGAGA	AAGGCGGACA	280
GGTATCCGGT	AAGCGGCAGG	GTCGGAACAG	GAGAGCGCAC	320
GAGGGAGCTT	CCAGGGGGAA	ACGCCTGGTA	TCTTTATAGT	360
CCTGTCGGGT	TTCGCCACCT	CTGACTTGAG	CGTCGATTTT	400
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CAGCAACGCG	GCC			453

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 209 base pairs



(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:3:

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GGATTCGAAC	CTTCGAAGTC	GATGACGGCA	GATTTAGAGT	80
CTGCTCCCTT	TGGCCGCTCG	GGAACCCAC	CACGGGTAAT	120
GCTTTTACTG	GCCTGCTCCC	TTATCGGGAA	GCGGGGCGCA	160
TCATATCAAA	TGACGCGCCG	CTGTAAAGTG	TTACGTTGAG	200
AAAGAATTC				209

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 209 base pairs

(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:4:

GAATTCTTTC	TCAACGTAAC	ACTTTACAGC	GGCGCGTCAT	40
TTGATATGAT	GCGCCCCGCT	TCCCGATAAG	GGAGCAGGCC	80
AGTAAAGCA	TTACCCGTGG	TGGGGTTCCC	GAGCGGCCAA	120
AGGGAGCAGA	CTCTAAATCT	GCCGTCATCG	ACTTCGAAGG	160
TTCGAATCCT	TCCCCACCA	CCATCACTTT	CAAAAGTCCG	200
AAAGAATTC				209

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:

AATAAA

6

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:6:

ATTAAA

6

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:7:

AGTAAA

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(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:8:

AAGAAC

6

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:9:

AATACA

6

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 227 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:10:

GCCTTAAGGG	CCATATGGTG	AGTGGATCCC	TTGACCCCAG	40
GCGGGGATGG	GGAGACCTGT	AGTCAGAGCC	CCCGGGCAGC	80
ACAGGCCAAT	GCCCGTCCTT	CCCCTGCAGG	ATGAGTAGTG	120
AGTGCCTCTC	CTGGCCCTGG	AAGTTGCCAC	TCCAGTGCCC	160
ACCAGCCTTG	TCCTAATAAA	ATTAAGTTGC	ATCATTTTGT	200
CTGACTAGGT	GTCCTCTATA	ATATTAT		227

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 227 base pairs
  - (B) TYPE: nucleic acid

(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:11:

ATAATATTAT	AGAGGACACC	TAGTCAGAAC	AAATGATGCA	40
ACTTAATTTT	ATTAGGACAA	GGCTGGTGGG	CACTGGAGTG	80
GCAACTTCCA	GGGCCAGGAG	AGGCACTCAC	TACTCATCCT	120
GCAGGGGAAG	GACGGGCATT	GGCCTGTGCT	GCCCGGGGGC	160
TCTGACTACA	GGTCTCCCC	ATCCCCGCCT	GGGGTCAAGG	200
CATCCACTCA	CCATATGGCC	CTTAAGG		227

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 252 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:12:

CCTCGGTACC	TGCCATGGCG	CGGATTCTTT	ATCACTGATA	40
AGTTGGTGGA	CATATTATGT	TTATCAGTGA	TAAAGTGTCA	80
AGCATGACAA	AGTTGCAGCC	GAATACAGTG	ATCCGTGCCG	120
GCCCTGGACT	GTTGAACGAG	GTCGGCGTAG	ACGGTCTGAC	160
GACACGCAAA	CTGGCGGAAC	GGTTGGGGGT	GCAGCAGCCG	200
GCGCTTTACT	GGCACTTCAG	GAACAAGCGG	GCGCCTTAAG	240
GGCCATATGC	CG			252

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 35 base pairs  
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:13:

CCTCGGTACC TGCCACCATG GCGCGGATTC TTTAT 35

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 38 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:14:

CGGCATATGG CCTTAAGGCG CCCGCTTGTT CCTGAAGT 38

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 228 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:15:

GCCTTAAGGG	CCATATGGTG	AGTGGATGCC	TTGACCCAG	40
GCGGGGATGG	GGGAGACCTG	TAGTCAGAGC	CCCCGGGCAG	80
CACAGGCCAA	TGCCCCTCCT	TCCCCTGCAG	GATGAGTAGT	120
GAGTGCCTCT	CCTGGCCCTG	GAAGTTGCCA	CTCCAGTGCC	160
CACCAGCCTT	GTCCTAATAA	AATTAAGTTG	CATCATTTTG	200
TCTGACTAGG	TGTCCTCTAT	AATATTAT		228

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1425 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:16:

TGCCATGGCG	CGGATTCTTT	ATCACTGATA	AGTTGGTGGA	40
CATATTATGT	TTATCAGTGA	TAAAGTGTCA	AGCATGACAA	80
AGTTGCAGCC	GAATACAGTG	ATCCGTGCCG	GCCCTGGACT	120
GTTGAACGAG	GTCGGCGTAG	ACGGTCTGAC	GACACGCAAA	160
CTGGCGGAAC	GGTTGGGGGT	GCAGCAGCCG	GCGCTTTACT	200
GGCACTTCAG	GAACAAGCGG	GCGCCTTAAG	GGCCATATGG	240
TGAGTGGATG	CCTTGACCCC	AGGCGGGGAT	GGGGGAGACC	280
TGTAGTCAGA	GCCCCCGGGC	AGCACAGGCC	AATGCCCGTC	320
CTTCCCCTGC	AGTGAGTAGT	GA CTGCCCGG	GTGGGATCCC	360
TGTGACCCCT	CCCCAGTGCC	TCTCCTGGCC	CTGGAAGTTG	400
CCACTCCAGT	GCCCACCAGC	CTTGTCCTAA	TAAAATTAAG	440
TTGCATCATT	TTGTCTGACT	AGGTGTCCTC	TATAATATTA	480
TAAGCTTGAT	ATCGAATTCT	TTCTCAACGT	AACACTTTAC	520
AGCGGCGCGT	CATTTGATAT	GATGCGCCCC	GCTTCCCGAT	560
AAGGGAGCAG	GCCAGTAAAA	GCATTACCCG	TGGTGGGGTT	600
CCCAGAGCGC	CAAAGGGAGC	AGACTCTAAA	TCTGCCGTCA	640
TCGACTTCGA	AGGTTCTGAAT	CCTTCCCCCA	CCACCATCAC	680
TTTCAAAAGT	CCGAAAGAAT	TCCTGCAGCC	CGTGTAGCCG	720
TAGTTAGGCC	ACCACTTCAA	GA ACTCTGTA	GCACCGCCTA	760
CATACCTCGC	TCTGCTAATC	CTGTTACCAG	TGGCTGCTGC	800
CAGTGGCGAT	AAGTCGTGTC	TTACCGGGTT	GGACTCAAGA	840
CGATAGTTAC	CGGATAAGGC	GCAGCGGTCG	GGCTGAACGG	880
GGGGTTTCGTG	CACACAGCCC	AGCTTGGAGC	GAACGACCTA	920
CACCGAACTG	AGATACCTAC	AGCGTGAGCA	TTGAGAAAGC	960
GCCACGCTTC	CCGAAGGGAG	AAAGGCGGAC	AGGTATCCGG	1000
TAAGCGGCAG	GGTCGGAACA	GGAGAGCGCA	CGAGGGAGCT	1040
TCCAGGGGGA	AACGCCTGGT	ATCTTTATAG	TCCTGTCGGG	1080
TTTCGCCACC	TCTGACTTGA	GCGTCGATTT	TTGTGATGCT	1120
CGTCAGGGGG	GCGGAGCCTA	TGGAAAAACG	CCAGCAACGC	1160
GGCCGGGGGA	TCCGGAGAGC	TCACTCTAGA	TGAGAGAGCA	1200
GTGAGGGAGA	GACAGAGACT	CGAATTTCCG	GAGCTATTTT	1240
AGTTTTCTTT	TCCGTTTTGT	GCAATTTTAC	TTATGATACC	1280
GGCCAATGCT	TGGTTGCTAT	TTTGGAAGCT	CCCCTTAGGG	1320
GATGCCCCTC	AACTGGCCCT	ATAAAGGGCC	AGCCTGAGCT	1360
GCAGAGGATT	CCTGCAGAGG	ATCAAGACAG	CACGTGGACC	1400
TCGCACAGCC	TCTCCACAG	GTACC		1425

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 719 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:17:

ATGAGCAAGG	GCGAGGAACT	GTTCACTGGC	GTGGTCCCAA	40
TTCTCGTGGA	ACTGGATGGC	GATGTGAATG	GGCACAAATT	80
TTCTGTCAGC	GGAGAGGGTG	AAGGTGATGC	CACATACGGA	120
AAGCTCACCC	TGAAATTCAT	CTGCACCACT	GGAAAGCTCC	160
CTGTGCCATG	GCCAACACTG	GTCACTACCT	TCACCTATGG	200
CGTGCAGTGC	TTTTCCAGAT	ACCCAGACCA	TATGAACGAG	240
CATGACTTTT	TCAAGAGCGC	CATGCCCGAG	GGCTATGTGC	280
AGGAGAGAAC	CATCTTTTTC	AAAGATGACG	GGAAC TACAA	320
GACCCGCGCT	GAAGTCAAGT	TCGAAGGTGA	CACCCTGGTG	360
AATAGAATCG	AGTTGAAGGG	CATTGACTTT	AAGGAAGATG	400
GAAACATTCT	CGGCCACAAG	CTGGAATACA	ACTATAACTC	440
CCACAATGTG	TACATCATGG	CCGACAAGCA	AAAGAATGGC	480
ATCAAGGTCA	ACTTCAAGAT	CAGACACAAC	ATTGAGGATG	520
GATCCGTGCA	GCTGGCCGAC	CATTATCAAC	AGAACACTCC	560
AATCGGCGAC	CGCCCTGTGC	TCCTCCCAGA	CAACAATTAC	600
CTGTCCACCC	AGTCTGCCCT	GTCTAAAGAT	CCCAACGAAA	640
AGAGAGACCA	CATGGTCCTG	CTGGAGTTTG	TGACCGCTGC	680
TGGGATCACA	CATGGCATGG	ACGAGCTGTA	CAAGTGAGC	719

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1911 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:18:

TATGAGCAAG	GGCGAGGAAC	TGTTCACTGG	CGTGGTCCCA	40
ATTCTCGTGG	AACTGGATGG	CGATGTGAAT	GGGCACAAAT	80
TTTCTGTCAG	CGGAGAGGGT	GAAGGTGATG	CCACATACGG	120
AAAGCTCACC	CTGAAATTCA	TCTGCACCAC	TGGAAAGCTC	160
CCTGTGCCAT	GGCCAACACT	GGTCACTACC	TTCACCTATG	200
GCGTGCAGTG	CTTTTCCAGA	TACCCAGACC	ATATGAAGCA	240
GCATGACTTT	TTCAAGAGCG	CCATGCCCGA	GGGCTATGTG	280
CAGGAGAGAA	CCATCTTTTT	CAAAGATGAC	GGGAACTACA	320
AGACCCGCGC	TGAAGTCAAG	TTCGAAGGTG	ACACCCTGGT	360
GAATAGAATC	GAGTTGAAGG	GCATTGACTT	TAAGGAAGAT	400
GGAAACATTTC	TCGGCCACAA	GCTGGAATAC	AACTATAACT	440
CCCACAATGT	GTACATCATG	GCCGACAAGC	AAAAGAATGG	480
CATCAAGGTC	AACTTCAAGA	TCAGACACAA	CATTGAGGAT	520
GGATCCGTGC	AGCTGGCCGA	CCATTATCAA	CAGAACACTC	560
CAATCGGCGA	CGGCCCTGTG	CTCCTCCAG	ACAACCATTA	600
CCTGTCCACC	CAGTCTGCCC	GTCTAAAGAT	CCCAACGAAA	640
AGAGAGACCA	CATGGTCCTG	CTGGAGTTTG	TGACCGCTGC	680
TGGGATCACA	CATGGCATGG	ACGAGCTGTA	CAAGTGAGCC	720
ATATGGTGAG	TGGATGCCTT	GACCCAGGC	GGGGATGGGG	760
GAGACCTGTA	GTCAGAGCCC	CCGGGCAGCA	CAGGCCAATG	800
CCCGTCCTTC	CCCTGCAGTG	AGTAGTGA	GCCCGGGTGG	840
GATCCCTGTG	ACCCCTCCCC	AGTGCCTCTC	CTGGCCCTGG	880
AAGTTGCCAC	TCCAGTGCCC	ACCAGCCTTG	TCCTAATAAA	920
ATTAAGTTGC	ATCATTTTGT	CTGACTAGGT	GTCTCTATA	960
ATATTATAAG	CTTGATATCG	AATTCTTTCT	CAACGTAACA	1000
CTTTACAGCG	GCGCGTCATT	TGATATGATG	CGCCCCGCTT	1040
CCCGATAAGG	GAGCAGGCCA	GTAAAAGCAT	TACCCGTGGT	1080
GGGGTTCCCG	AGCGGCCAAA	GGGAGCAGAC	TCTAAATCTG	1120
CCGTCATCGA	CTTCGAAGGT	TCGAATCCTT	CCCCCACCAC	1160
CATCACTTTC	AAAAGTCCGA	AAGAATTCCT	GCAGCCCGTG	1200
TAGCCGTAGT	TAGGCCACCA	CTTCAAGAAC	TCTGTAGCAC	1240
CGCCTACATA	CCTCGCTCTG	CTAATCCTGT	TACCAGTGGC	1280
TGCTGCCAGT	GGCGATAAGT	CGTGTCTTAC	CGGGTTGGAC	1320
TCAAGACGAT	AGTTACCGGA	TAAGGCGCAG	CGGTCGGGCT	1360
GAACGGGGGG	TTCGTGCACA	CAGCCCAGCT	TGGAGCGAAC	1400
GACCTACACC	GAAGTGAAGT	ACCTACAGCG	TGAGCATTGA	1440
GAAAGCGCCA	CGCTTCCCGA	AGGGAGAAAG	GCGGACAGGT	1480
ATCCGGTAAG	CGGCAGGGTC	GGAACAGGAG	AGCGCACGAG	1520
GGAGCTTCCA	GGGGGAAACG	CCTGGTATCT	TTATAGTCCT	1560
GTCGGGTTTC	GCCACCTCTG	ACTTGAGCGT	CGATTTTTGT	1600
GATGCTCGTC	AGGGGGGCGG	AGCCTATGGA	AAAACGCCAG	1640
CAACGCGGCC	GGGGGATCCG	GAGAGCTCAC	TCTAGATGAG	1680
AGAGCAGTGA	GGGAGAGACA	GAGACTCGAA	TTTCCGGAGC	1720
TATTTTCAGTT	TTCTTTTCCG	TTTTGTGCAA	TTTCACTTAT	1760
GATACCGGCC	AATGCTTGGT	TGCTATTTTG	GAAACTCCCC	1800
TTAGGGGATG	CCCCTCAACT	GGCCCTATAA	AGGGCCAGCC	1840
TGAGCTGCAG	AGGATTCCTG	CAGAGGATCA	AGACAGCACG	1880
TGGACCTCGC	ACAGCCTCTC	CCACAGGTAC	C	1911

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 69 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown



(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:19:

Pro Asp Leu Ser Tyr Met Pro Ile Trp Lys Phe Pro  
1 5 10  
Asp Glu Glu Gly Ala Cys Gln Pro Cys Pro Ile Asn  
15 20  
Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys Gly  
25 30 35  
Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser  
40 45  
Ile Ile Ser Ala Val Val Gly Ile Leu Leu Val Val  
50 55 60  
Val Leu Gly Val Val Phe Gly Ile Leu  
65

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 287 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:20:

Pro Ala Pro Gly Ala Gly Gly Met Val His His Arg  
1 5 10  
His Arg Ser Ser Ser Thr Arg Ser Gly Gly Gly Asp  
15 20  
Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu Ala  
25 30 35  
Pro Arg Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly  
40 45  
Ser Asp Val Phe Asp Gly Asp Leu Gly Met Gly Ala  
50 55 60  
Ala Lys Gly Leu Ser Leu Pro Thr His Asp Pro Ser  
65 70  
Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Val Pro  
75 80  
Leu Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu  
85 90 95  
  
Thr Cys Ser Pro Gln Pro Glu Tyr Val Asn Gln Pro  
100 105  
Asp Val Arg Pro Pro Pro Ser Pro Arg Glu Gly Pro  
110 115 120  
  
Leu Pro Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu  
125 130  
Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val  
135 140

Val	Lys	Asp	Val	Phe	Ala	Phe	Gly	Gly	Ala	Val	Glu
145					150					155	
Asn	Pro	Glu	Tyr	Leu	Thr	Pro	Gln	Gly	Thr	Cys	Ser
		160						165			
Pro	Gln	Pro	Glu	Tyr	Val	Asn	Gln	Pro	Asp	Val	Arg
	170					175					180
Pro	Gln	Pro	Pro	Ser	Pro	Arg	Glu	Gly	Pro	Leu	Pro
				185					190		
Ala	Ala	Arg	Pro	Ala	Gly	Ala	Thr	Leu	Glu	Arg	Pro
		195					200				
Lys	Leu	Ser	Pro	Gly	Lys	Asn	Gly	Val	Val	Lys	Asp
205					210					215	
Val	Phe	Ala	Phe	Gly	Gly	Ala	Val	Glu	Asn	Pro	Glu
			220					225			
Tyr	Leu	Thr	Pro	Gln	Gly	Gly	Ala	Ala	Pro	Gln	Pro
	230					235					240
His	Pro	Pro	Pro	Ala	Phe	Ser	Pro	Ala	Phe	Asp	Asn
				245					250		
Leu	Tyr	Tyr	Trp	Asp	Asp	Pro	Pro	Glu	Arg	Gly	Ala
		255					260				
Pro	Pro	Ser	Thr	Phe	Lys	Gly	Thr	Pro	Thr	Ala	Glu
260					270					275	
Asn	Pro	Glu	Tyr	Leu	Gly	Leu	Asp	Val	Pro	Val	
			280					285			

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:21:

Ile	Ile	Ser	Ala	Val	Val	Gly	Ile	Leu	Leu	Val	Val
1				5					10		
Val	Leu	Gly	Val	Val	Phe	Gly	Ile	Leu	Ile		
		15					20				

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2125 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:22:

GCCACCATGG	CCCCTGACCT	CTCCTACATG	CCCATCTGGA	40
AGTTTTCAGA	TGAGGAGGGC	GCATGCCAGC	CTTGCCCCAT	80
CAACTGCACC	CACTCCTGTG	TGGACCTGGA	TGACAAGGGC	120
TGCCCCGCCG	AGCAGAGAGC	CAGCCCTCTG	ACGTCCATCA	160
TCTCTGCGGT	GGTTGGCATT	CTGCTGGTCG	TGGTCTTGGG	200
GGTGGTCTTT	GGGATCCTCA	TCAAGCGACG	GCAGCAGAAG	240
ATCACATGTC	CAGACCCTGC	CCCGGGCGCT	GGGGGCATGG	280
TCCACCACAG	GCACCGCAGC	TCATCTACCA	GGAGTGGCGG	320
TGGGGACCTG	ACACTAGGGC	TGGAGCCCTC	TGAAGAGGAG	360
GCCCCCAGGT	CTCCACTGGC	ACCCTCCGAA	GGGGCTGGCT	400
CCGATGTATT	TGATGGTGAC	CTGGGAATGG	GGGCAGCCAA	440
GGGGCTGCAA	AGCCTCCCCA	CACATGACCC	CAGCCCTCTA	480
CAGCGGTACA	GTGAGGACCC	CACAGTACCC	CTGCCCTCTG	520
AGACTGATGG	CTACGTTGCC	CCCCTGACCT	GCAGCCCCCA	560
GCCTGAATAT	GTGAACCAGC	CAGATGTTTCG	GCCCCAGCCC	600
CCTTCGCCCC	GAGAGGGCCC	TCTGCCTGCT	GCCCCGACCTG	640
CTGGTGCCAC	TCTGGAAAGG	CCCAAGACTC	TCTCCCCAGG	680
GAAGAATGGG	GTCGTCAAAG	ACGTTTTTGC	CTTTGGGGGT	720
GCCGTGGAGA	ACCCCAGAGC	TTGACACCCC	AGGGAGGAGC	760
TGCCCCCTCAG	CCCCACCCTC	CTCCTGCCTT	CAGCCCAGCC	800
TTCGACAACC	TCTATTACTG	GGACCAGGAC	CCACCAGAGC	840
GGGGGGCTCC	ACCAGCACC	TTCAAAGGGA	CACCTACGGC	880
AGAGAACCCA	GAGTACCTGG	GTCTGGACGT	GCCAGTGTGA	920
AGCCTTAAGG	GCCATATGGT	GAGTGGATGC	CTTGACCCCA	960
GGCGGGGATG	GGGGAGACCT	GTAGTCAGAG	CCCCCGGGCA	1000
GCACAGGCCA	ATGCCCGTCC	TTCCCCTGCA	GTGAGTAGTG	1040
ACTGCCCGGG	TGGGATCCCT	GTGACCCCTC	CCCAGTGCCT	1080
CTCCTGGCCC	TGGAAGTTGC	CACTCCAGTG	CCCACCAGCC	1120
TTGTCCTAAT	AAAATTAAGT	TGCATCATTT	TGTCTGACTA	1160
GGTGTCTCT	ATAATATTAT	AAGCTTGATA	TCGAATTCTT	1200
TCTCAACGTA	ACACTTTACA	GCGGCGCGTC	ATTTGATATG	1240
ATGCGCCCCG	CTTCCCGATA	AGGGAGCAGG	CCAGTAAAAG	1280
CATTACCCGT	GGTGGGGTTC	CCGAGCGGCC	AAAGGGAGCA	1320
GACTCTAAAT	CTGCCGTCAT	CGACTTCGAA	GGTTCGAATC	1360
CTTCCCCCAC	CACCATCACT	TTCAAAGGTC	CGAAAGAATT	1400
CCTGCAGCCC	GTGTAGCCGT	AGTTAGGCCA	CCACTTCAAG	1440
AACTCTGTAG	CACCGCCTAC	ATACCTCGCT	CTGCTAATCC	1480
TGTTACCAGT	GGCTGCTGCC	AGTGGCGATA	AGTCGTGTCT	1520
TACCGGGTTG	GACTCAAGAC	GATAGTTACC	GGATAAGGCG	1560
CAGCGGTCGG	GCTGAACGGG	GGGTTCGTGC	ACACAGCCCA	1600
GCTTGAGAGC	AACGACCTAC	ACCGAACTGA	GATACCTACA	1640
GCGTGAGCAT	TGAGAAAGCG	CCACGCTTCC	CGAAGGGAGA	1680
AAGGCGGACA	GGTATCCGGT	AAGCGGCAGG	GTGGAACAG	1720
GAGAGCGCAC	GAGGGAGCTT	CCAGGGGGAA	ACGCCTGGTA	1760
TCTTTATAGT	CCTGTCGGGT	TTCGCCACCT	CTGACTTGAG	1800
CGTCGATTTT	TGTGATGCTC	GTGAGGGGGG	CGGAGCCTAT	1840
GGAAAAACGC	CAGCAACGCG	GCCGGGGGAG	CCGGAGAGCT	1880
CACTCTAGAT	GAGAGAGCAG	TGAGGGAGAG	ACAGAGACTC	1920
GAATTTCCGG	AGCTATTTCA	GTTTTCTTTT	CCGTTTTGTG	1960

CAATTTCACT	TATGATACCG	GCCAATGCTT	GGTTGCTATT	2000
TTGGAAACTC	CCCTTAGGGG	ATGCCCCTCA	ACTGGCCCTA	2040
TAAAGGGCCA	GCCTGAGCTG	CAGAGGATTC	CTGCAGAGGA	2080
TCAAGACAGC	ACGTGGACCT	CGCACAGCCT	CTCCCACAGG	2120
TACCT				2125

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: oligonucleotide
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:23:

GTCTGCCACC ATGGCCTACT CCCCTGC 27

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: oligonucleotide
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:24:

TTCTTTGGTG ACCTACCTCT TCGGAATTGC CGAGTC 36

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1242 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:25:

ATGGAGGAGC CGCAGTCAGA TCCTAGCGTC GAGCCCCCTC 40  
TGAGTCAGGA AACATTTTCA GACCTATGGA AACTACTTCC 80  
TGAAAACAAC GTTCTGTCCC CTTGCGCGTC CCAAGCAATG 120  
GATGATTTGA TGCTGTCCCC GGACGATATT GAACAATGGT 160  
TCACTGAAGA CCCAGGTCCA GATGAAGCTC CCAGAATGCC 200  
AGAGGCTGCT CCCCGCGTGG CCCCTGCACC AGCAGCTCCT 240  
ACACCGGCGG CCCCTGCACC AGCCCCCTCC TGGCCCCCTG 280  
CATCTTCTGT CCCTTCCCAG AAAACCTACC AGGGCAGCTA 320  
CGGTTTCCGT CTGGGCTTCT TGCATTCTGG GACAGCCAAG 360  
TCTGCCACCA TGGCCTACTC CCCTGCGTCT GTGACTTGCA 400  
CGTACTCCCC TGCCCTCAAC AAGATGTTTT GCCAACTGGC 440  
CAAGACCTGC CCTGTGCAGC TGTGGGTTGA TTCCACACCC 480  
CCGCCCAGCA CCCGCGTCCG CGCCATGGCC ATCTACAAGC 520  
AGTCACAGCA CATGACGGAG GTTGTGAGGC GCTGCCCCCA 560  
CCATGAGCGC TGCTCAGATA GCGATGGTCT GGCCCCCTCT 600  
CAGCGTCTTA TCCGAGTGGA AGGAAATTTG CGTGTGGAGT 640  
ATTTGGATGA CAGAAACACT TTTCGACATA GTGTGGTGGT 680  
GCCCTATGAG CCGCCTGAGG TTGGCTCTGA CTGTACCACC 720  
ATCCACTACA ACTACATGTG TAACAGTTCC TGCATGGGCG 760  
GCATGAACCG GAGGCCCATC CTCACCATCA TCACACTGGA 800  
AGACTCCAGT GGTAATCTAC TGGGACGGAA CAGCTTTGAG 840  
GTGCGTGTTT GTGCCTGTCC TGGGAGAGAC CGGCGCACAG 880  
AGGAAGAGAA TCTCCGCAAG AAAGGGGAGC CTCACCACGA 920  
GCTGCCCCCA GGGAGCACTA AGCGAGCATA GCCCAACAAC 960  
ACCAGCTCCT CTCCCCAGCC AAAGAAGAAA CCACTGGATG 1000  
GAGAATATTT CACCCTTCAG ATCCGTGGGC GTGAGCGCTT 1040  
CGAGATGTTT TTTGGTGACC TACCTCTTCG GAATTGCCGA 1080  
GTCTTCCGAG AGCTGAATGA GGCCTTGGAA CTCAAGGATG 1120  
CCCAGGCTGG GAAGGAGCCA GGGGGGAGCA GGGCTCACTC 1160  
CAGCCACCTG AAGTCCAAAA AGGGTCAGTC TACCTCCCGC 1200  
CATAAAAAAC TCATGTTCAA GACAGAAGGG CCTGACTCAG 1240  
AC

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 608 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:26:

CTCGGGCCGC	GTTGCTGGCG	TTTTTCCATA	GGCTCCGCCC	40
CCCTGACGAG	CATCACAAAA	ATCGACGCTC	AAGTCAGAGG	80
TGGCGAAACC	CGACAGGACT	ATAAAGATAC	CAGGCGTTTC	120
CCCCTGGAAG	CTCCCTCGTG	CGCTCTCCTG	TTCCGACCCT	160
GCCGCTTACC	GGATACCTGT	CCGCCTTTCT	CCCTTCGGGA	200
AGCGTGCGC	TTTCTCAATG	CTCACGCTGT	AGGTATCTCA	240
GTTGCGGTGA	GGTCGTTTCG	TCCAAGCTGG	GCTGTGTGCA	280
CGAACCCCCC	GTTCAGCCCG	ACCGCTGCGC	CTTATCCGGT	320
AACTATCGTC	TTGAGTCCAA	CCCGGTAAGA	CACGACTTAT	360
CGCCACTGGC	AGCAGCCACT	GGTAACAGGA	TTAGCAGAGC	400
GAGGTATGTA	GGCGGTGCTA	CAGAGTTCTT	GAAGTGGTGG	440
CCTAACTACG	GCTACACTAG	AAGGACAGTA	TTTGGTATCT	480
GCGCTCTGCT	GAAGCCAGTT	ACCTTCGGAA	AAAGAGTTGG	520
TAGCTCTTGA	TCCGGCAAAC	AAACCACCGC	TGGTAGCGGT	560
GGTTTTTTTG	TTTGCAAGCA	GCAGATTACG	CGCAGAAAAA	600
AAGGATCT				608

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1547 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:27:

GGTACCTGCC	ACCATGGCGC	GGATTCTTTA	TCACTGATAA	40
GTTGGTGGAC	ATATTATGTT	TATCAGTGAT	AAAGTGTCAA	80
GCATGACAAA	GTTGCAGCCG	AATACAGTGA	TCCGTGCCGG	120
CCCTGGACTG	TTGAACGAGG	TCGGCGTAGA	CGGTCTGACG	160
ACACGCAAAC	TGGCGGAACG	GTTGGGGGTG	CAGCAGCCGG	200
CGCTTTACTG	GCACTTCAGG	AACAAGCGGG	CGCCTTAAGG	240
GCCATATGGT	GAGTGGATGC	CTTGACCCCA	GGCGGGGATG	280
GGGGAGACCT	GTAGTCAGAG	CCCCCGGGCA	GCACAGGCCA	320
ATGCCCCGCC	TTCCCCTGCA	GGATGAGTAG	TGAGTGCCTC	360
TCCTGGCCCT	GGAAGTTGCC	ACTCCAGTGC	CCACCAGCCT	400
TGTCCTAATA	AAATTAAGTT	GCATCATTTT	GTCTGACTAG	440
GTGTCCTCTA	TAATATTATA	AGCTTGATAT	CGAATTCTTT	480
CGGACTTTTG	AAAGTGATGG	TGGTGGGGGA	AGGATTGCAA	520
CCTTCGAAGT	CGATGACGGC	AGATTTAGAG	TCTGCTCCCT	560
TTGGCCGCTC	GGAACCCCA	CCACGGGTAA	TGCTTTTACT	600
GGCCTGCTCC	CTTATCGGGA	AGCGGGGCGC	ATCATATCAA	640
ATGACGCGCC	GCTGTAAAGT	GTTACGTTGA	GAAAGAATTC	680
CTGCAGCCCC	CCGCGTTGCT	GGCGTTTTTC	CATAGGCTCC	720

GCCCCCTGA	CGAGCATCAC	AAAAATCGAC	GCTCAAGTCA	760
GAGGTGGCGA	AACCCGACAG	GACTATAAAG	ATACCAGGCG	800
TTTCCCCCTG	GAAGCTCCCT	CGTGCGCTCT	CCTGTTCCGA	840
CCCTGCCGCT	TACCGGATAC	CTGTCCGCCT	TTCTCCCTTC	880
GGGAAGCGTG	GCGCTTTCTC	AATGCTCACG	CTGTAGGTAT	920
CTCAGTTCGG	TGTAGGTCGT	TCGCTCCAAG	CTGGGCTGTG	960
TGCACGAACC	CCCCGTTTCA	CCCGACCGCT	GCGCCTTATC	1000
CGGTAACAT	CGTCTTGAGT	CCAACCCGGT	AAGACACGAC	1040
TTATCGCCAC	TGGCAGCAGC	CACTGGTAAC	AGGATTAGCA	1080
GAGCGAGGTA	TGTAGGCGGT	GCTACAGAGT	TCTTGAAGTG	1120
GTGGCCTAAC	TACGGCTACA	CTAGAAGGAC	AGTATTTGGT	1160
ATCTGCGCTC	TGCTGAAGCC	AGTTACCTTC	GGAAAAAGAG	1200
TTGGTAGCTC	TTGATCCGGC	AAACAAACCA	CCGCTGGTAG	1240
CGGTGGTTTT	TTTGTTTGCA	AGCAGCAGAT	TACGCGCAGA	1280
AAAAAAGGAT	CTGGGGGATC	CGGAGAGCTC	ACTCTAGATG	1320
AGAGAGCAGT	GAGGGAGAGA	CAGAGACTCG	AATTTCCGGA	1360
GCTATTTTCA	TTTTCTTTTC	CGTTTTGTGC	AATTTCACTT	1400
ATGATACCGG	CCAATGCTTG	GTTGCTATTT	TGGAAACTCC	1440
CCTTAGGGGA	TGCCCCCTCA	CTGGCCCTAT	AAAGGGCCAG	1480
CCTGAGCTGC	AGAGGATTCC	TGCAGAGGAT	CAAGACAGCA	1520
CGTGGACCTC	GCACAGCCTC	TCCCACA		1547

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1807 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GGTACCTGCC	ACCATGGCGC	GGATTCTTTA	TCACTGATAA	40
GTTGGTGGAC	ATATTATGTT	TATCAGTGAT	AAAGTGTCAA	80
GCATGACAAA	GTTGCAGCCG	AATACAGTGA	TCCGTGCCGG	120
CCCTGGACTG	TTGAACGAGG	TCGGCGTAGA	CGGTCTGACG	160
ACACGCAAA	TGGCGGAACG	GTTGGGGGTG	CAGCAGCCGG	200
CGCTTTACTG	GCACTTCAGG	AACAAGCGGG	CGCCTTAAGG	240
GCCATATGGT	GAGTGGATGC	CTTGACCCCA	GGCGGGGATG	280
GGGGAGACCT	GTAGTCAGAG	CCCCCGGGCA	GCACAGGCCA	320
ATGCCCGTCC	TTCCCCTGCA	GGATGAGTAG	TGAGTGCCTC	360
TCCTGGCCCT	GGAAGTTGCC	ACTCCAGTGC	CCACCAGCCT	400
TGTCCTAATA	AAATTAAGTT	GCATCATTTT	GTCTGACTAG	440
GTGTCCTCTA	TAATATTATA	AGCTTGATAT	CGAATTCTTT	480
CGGACTTTTG	AAAGTGATGG	TGGTGGGGGA	AGGATTTCGA	520
CCTTCGAAGT	CGATGACGGC	AGATTTAGAG	TCTGCTCCCT	560
TTGGCCGCTC	GGGAACCCCA	CCACGGGTAA	TGCTTTTACT	600
GGCCTGCTCC	CTTATCGGGA	AGCGGGGCGC	ATCATATCAA	640
ATGACGCGCC	GCTGTAAAGT	GTTACGTTGA	GAAAGAATTC	680

CTGCAGCCCCG	CCGCGTTGCT	GGCGTTTTTC	CATAGGCTCC	720
GCCCCCTGA	CGAGCATCAC	AAAAATCGAC	GCTCAAGTCA	760
GAGGTGGCGA	AACCCGACAG	GAATAAAAG	ATACCAGGCG	800
TTTCCCCCTG	GAAGCTCCCT	CGTGCGCTCT	CCTGTTCCGA	840
CCCTGCCGCT	TACCGGATAC	CTGTCCGCCT	TTCTCCCTTC	880
GGGAAGCGTG	GCGCTTTCTC	AATGCTCACG	CTGTAGGTAT	920
CTCAGTTCGG	TGTAGGTCGT	TCGCTCCAAG	CTGGGCTGTG	960
TGCACGAACC	CCCCGTTTCA	CCCGACCGCT	GCGCCTTATC	1000
CGGTAACTAT	CGTCTTGAGT	CCAACCCGGT	AAGACACGAC	1040
TTATCGCCAC	TGGCAGCAGC	CACTGGTAAC	AGGATTAGCA	1080
GAGCGAGGTA	TGTAGGCGGT	GCTACAGAGT	TCTTGAAGTG	1120
GTGGCCTAAC	TACGGCTACA	CTAGAAGGAC	AGTATTTGGT	1160
ATCTGCGCTC	TGCTGAAGCC	AGTTACCTTC	GGAAAAAGAG	1200
TTGGTAGCTC	TTGATCCGCG	AAACAAACCA	CCGCTGGTAG	1240
CGGTGGTTTT	TTTGTTTGCA	AGCAGCAGAT	TACGCGCAGA	1280
AAAAAAGGAT	CTGGGGGATC	CGGAGAGCTC	CCAACGCGTT	1320
GGATGCATGG	ATGAGGGAAA	GGAGGTAAGA	TCTGTAATGA	1360
ATAAGCAGGA	ACTTTGAAGA	CTCAGTGACT	CAGTGAGTAA	1400
TAAAGACTCA	GTGACTTCTG	ATCCTGTCTT	AACTGCCACT	1440
CCTTGTTGTC	CCAAGAAAGC	GGCTTCCTGC	TCTCTGAGGA	1480
GGACCCCTTC	CCTGGAAGGT	AAAATAAGG	ATGTCAGCAG	1520
AGAAATTTTT	CCACCATTGG	TGCTTGGTCA	AAGAGGAAAC	1560
TGATGAGCTC	ACTCTAGATG	AGAGAGCAGT	GAGGGAGAGA	1600
CAGAGACTCG	AATTTCCGGA	GCTATTTTCA	TTTTCTTTTC	1640
CGTTTTGTGC	AATTTCACTT	ATGATAACCG	CCAATGCTTG	1680
GTTGCTATTT	TGGAAACTCC	CCTTAGGGGA	TGCCCCTCAA	1720
CTGGCCCTAT	AAAGGGCCAG	CCTGAGCTGC	AGAGGATTCC	1760
TGCAGAGGAT	CAAGACAGCA	CGTGGACCTC	GCACAGCCTC	1800
TCCCACA				1807

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2308 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:29:

GGTACCTGCC	ACCATGGCGA	AGGGCGAGGA	ACTGTTCACT	40
GGCGTGGTCC	CAATTCTCGT	GGAAGTGGAT	GGCGATGTGA	80
ATGGGCACAA	ATTTTCTGTC	AGCGGAGAGG	GTGAAGGTGA	120
TGCCACATAC	GGAAAGCTCA	CCCTGAAATT	CATCTGCACC	160
ACTGGAAAGC	TCCCTGTGCC	ATGGCCAACA	CTGGTCACTA	200
CCTTCACCTA	TGGCGTGCAG	TGCTTTTCCA	GATACCCAGA	240
CCATATGAAG	CAGCATGACT	TTTTCAAGAG	CGCCATGCCC	280



GAGGGCTATG	TGCAGGAGAG	AACCATCTTT	TTCAAAGATG	320
ACGGGAAC TA	CAAGACCCGC	GCTGAAGTCA	AGTTCGAAGG	360
TGACACCCTG	GTGAATAGAA	TCGAGTTGAA	GGGCATTGAC	400
TTTAAGGAAG	ATGGAAACAT	TCTCGGCCAC	AAGCTGGAAT	440
ACA ACTATAA	CTCCCACAAT	GTGTACATCA	TGGCCGACAA	480
GCAAAAAGAAT	GGCATCAAGG	TCAACTTCAA	GATCAGACAC	520
AACATTGAGG	ATGGATCCGT	GCAGCTGGCC	GACCATTTATC	560
AACAGAACAC	TCCAATCGGC	GACGGCCCTG	TGCTCCTCCC	600
AGACAACCAT	TACCTGTCCA	CCCAGTCTGC	CCTGTCTAAA	640
GATCCCAACG	AAAAGAGAGA	CCACATGGTC	CTGCTGGAGT	680
TTGTGACCGC	TGCTGGGATC	ACACATGGCA	TGGACGAGCT	720
GTACAAGTGA	GCGCCTTAAG	GGCCATATGG	TGAGTGATG	760
CCTTGACCCC	AGGCGGGGAT	GGGGGAGACC	TGTAGTCAGA	800
GCCCCCGGGC	AGCACAGGCC	AATGCCCGTC	CTTCCCTGTC	840
AGGATGAGTA	GTGAGTGCCT	CTCCTGGCCC	TGGAAGTTGC	880
CACTCCAGTG	CCCACCAGCC	TTGTCCTAAT	AAAATTAAGT	920
TGCATCATTT	TGTCTGACTA	GGTGTCTCT	ATAATATTAT	960
AAGCTTGATA	TCGAATTCTT	TCGGACTTTT	AAAAGTGATG	1000
GTGGTG GGGG	AAGGATTCGA	ACCTTCGAAG	TCGATGACGG	1040
CAGATTTAGA	GTCTGCTCCC	TTTGGCCGCT	CGGGAACCCC	1080
ACCACGGGTA	ATGCTTTTAC	TGGCCTGCTC	CCTTATCGGG	1120
AAGCGGGGCG	CATCATATCA	AATGACGCGC	CGCTGTAAAG	1160
TGTTACGTTG	AGAAAGAATT	CCTGCAGCCC	GCCGCGTTGC	1200
TGGCGTTTTT	CCATAGGCTC	CGCCCCCTGC	ACGAGCATCA	1240
CAAAAAATCGA	CGCTCAAGTC	AGAGGTGGCG	GAACCCGACA	1280
GGACTATAAA	GATACCAGGC	GTTTCCCCCT	GGAAGCTCCC	1320
TCGTGCGCTC	TCCTGTTCCG	ACCCTGCCGC	TTACCCGATA	1360
CCTGTCCGCC	TTTCTCCCTT	CGGGAAGCGT	GGCGCTTTCT	1400
CAATGCTCAC	GCTGTAGGTA	TCTCAGTTCG	GTGTAGGTCG	1440
TTCGCTCCAA	GCTGGGCTGT	GTGCACGAAC	CCCCCGTTCA	1480
GCCCGACCGC	TAGGCCTTAT	CCGGTAAC TA	TCGTCTTGAG	1520
TCCAAACCCG	TAAGACACGA	CTTATCGCCA	CTGGCAGCAG	1560
CCACTGGTAA	CAGGATTAGC	AGAGCGAGGT	ATGTAGGCGG	1600
TGCTACAGAG	TTCTTGAAGT	GGTGGCCTAA	CTACGGCTAC	1640
ACTAGAAGGA	CAGTATTTGG	TATCTGCGCT	CTGCTGAAGC	1680
CAGTTACCTT	CGGAAAAAGA	GTTGGTAGCT	CTTGATCCGG	1720
CAAACAAACC	ACCGCTGGTA	GCGGTGGTTT	TTTTGTTTGC	1760
AAGCAGCAGA	TTACGCGCAG	AAAAAAAGGA	TCTGGGGGAT	1800
CCGGAGAGCT	CCCAACGCGT	TGGATGCATG	GATGAGGGAA	1840
AGGAGGTAAG	ATCTGTAATG	AATAAGCAGG	AACTTTGAAG	1880
ACTCAGTGAC	TCAGTGAGTA	ATAAAGACTC	AGTGACTTCT	1920
GATCCTGTCC	TAAGTCCAC	TCCTTGTTGT	CCCAAGAAAG	1960
CGGCTTCCTG	CTCTCTGAGG	AGGACCCCTT	CCCTGGAAGG	2000
TAAACTAAG	GATGTCAGCA	GAGAAATTTT	TCCACCATTG	2040
GTGCTTGGTC	AAAGAGGAAA	CTGATGAGCT	CACTCTAGAT	2080
GAGAGAGCAG	TGAGGGAGAG	ACAGAGACTC	GAATTTCCGG	2120
AGCTATTTCA	GTTTTCTTTT	CCGTTTTGTG	CAATTTCACT	2160
TATGATACCG	GCCAATGCTT	GGTTGCTATT	TTGGAAACTC	2200
CCCTTAGGGG	ATGCCCTCA	ACTGGCCCTA	TAAAGGGCCA	2240
GCCTGAGCTG	CAGAGGATTC	CTGCAGAGGA	TCAAGACAGC	2280
ACGTGGACCT	CGCACAGCCT	CTCCCACA		2308

FIG. 1

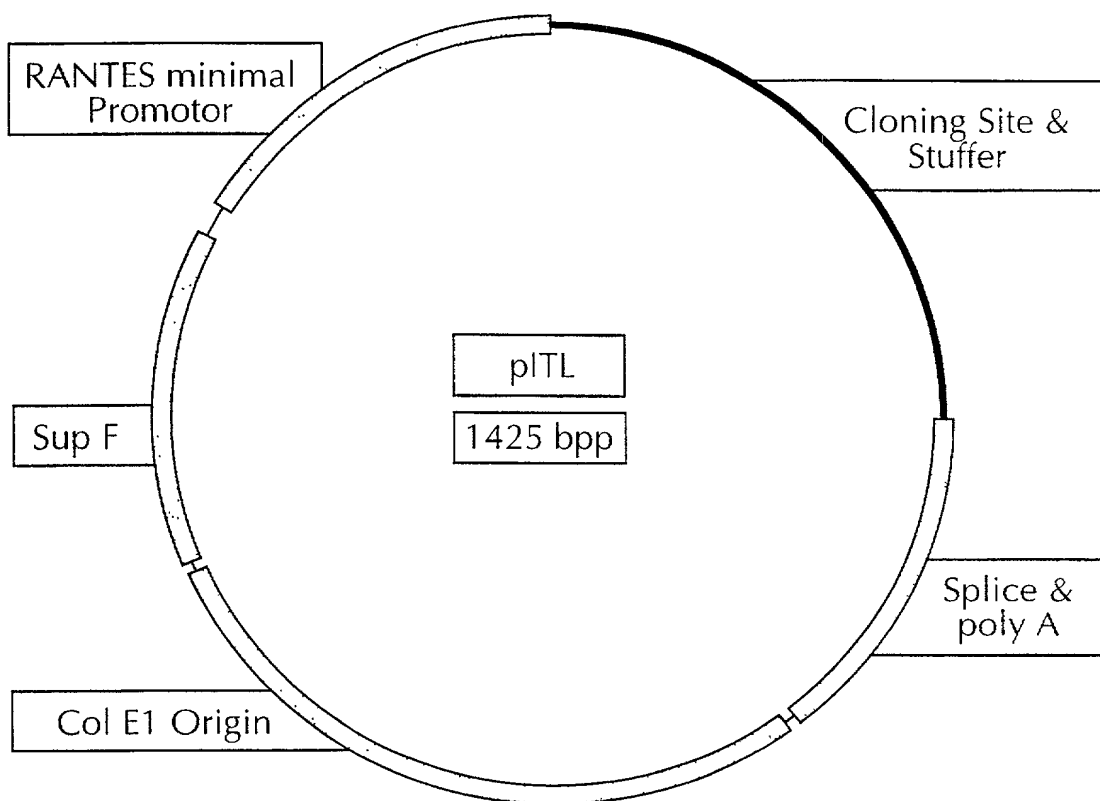
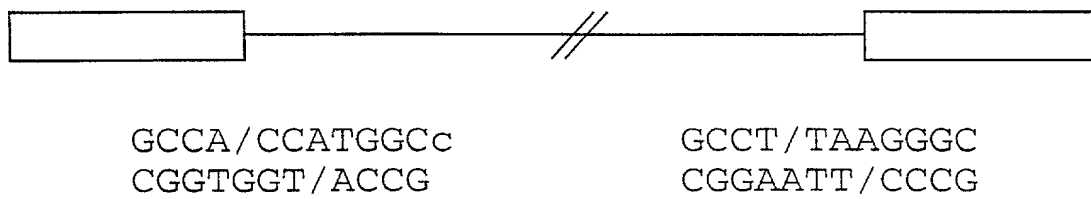


FIG. 2



# FIG. 3A

MRPSGTAGAALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLSLQRMFNNCVVLNLEITYVQRNYD  
M---ELAALCRWGLLALLP-PGA-AST-VCTGTDMLRLPASPETHLDMLRHL YQGCQVQGNLELTYLPTNAS  
60 57

LSFLKTIQEVAGYVLIAlNTVERIPLENLQIIRGNMYYSYALAVLSNYD--AN-----GLKELPMRNL  
125 140  
LSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTLQFEDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSL  
122 147

QEILHGAVRFSNNPALCNVESIQWRDIVSSDFLSNMSMDFQNHLSGCQKCDPSCPNGS-CWGAGEENCQKLTkII  
175 \* \* \* \* \*  
TEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQALTLIDTNRSRACHPCSPMCK-GSRCWGESSEDCQSLTRTV  
175

CAQCSCGRGKSPSDCCCHNQCAAGCTGPRESDECLVCRKFRDEATCKDTCPPMLLYNPITYQMDVNPEGKYSFGA  
289  
CAGGCA-RCKGPLPTDCCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVITYNTDTFESMPNPEGRYTFGA  
295

YVVTDHGSCVRACGADSYEME-EDGVRKCKKCEGPCRKVCNCGIGIGEFKDSLSINATNIKHFKNCTISIGDLLHIL  
350 \*  
YLSTDVGSCTLVCPLHNQEVTAEDGTQRCCKCSKPCARVCYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFL  
352

PVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQPGQFSLAVVSLNITS  
438  
PESFDGDPASNTAPLQPEQLQVFETLEEITGYLYISAWPDSLPDLSVFQNLQVIRGRILHNHNGAYSLTLQGLGISW  
445

**FIG. 3B**

LGLRSLKEISDGDVIIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCGWGPEDR  
|||||: : |||: : |||: : |||: : |||: : |||: :  
LGLRSLRELGSGLAIHHNTHLCFVHTVPWDQLFRNP HQALLHTANRPEDECVGEGLACHQLCARGHCWGPGPTQ  
500

CVSCRNVSRGRECVCKLLEGEPRFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPA  
CVNCSQFLRGQECVEECRVLQGLPREYVNAHCLPCHPECPQNGSVTCFGPEADQCVCACHYKDPFCVARCPS  
531 583 595

GVMGENNTLV-WKYADAGHVCHLCHPNCYTGCTGPGLEGCPTNGPKIPSIATGMVGALLLLVVAL--GIGLF  
||| : ||| | | | | | | | | | | : |||  
GVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVDLDKGP AEQRASPLTSII SAVVGILLVVVLGVVFGI-LLI

631                  \*        \*        \*        \*        \*

644

[illegible]

775

VAIKELREATSPKANKEILDEAYVMASVDNPHVCRLLGICLTSTVQLITQLMPPFGCLLDYVREHKDNIGSQYLIN  
||||| ||||| ||||| ||||| ||||| : ||||| : |||||  
VAIKVLRENTSPKANKEILDEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLMMPYGCLLDHV TENRGR LGSQDLIN

790

	850	877
WCVQIAKGMNYLEDVRLAARNVLVKTPQHVKITDFGLAKLLGAEKEEYHAECCGVPIKWMALESILHRIV		
:                         :		
WCMQIAKGMSYLEDVRLVHRDLAARNLVKSPNHVKITDFGLARLDDIDETEYHADGGKVPIKWMALESILRRRF		
	850	892

**FIG. 3C**

929

THQSDVWSYGVTVWELMTFGAKPYDGI  
PAREIPDLLEKGERLPQPPICTIDVYMIMVKCWMIDSECRPRFRELVS  
940

1010

990

[illegible]

```

-----SSPSTSRTPLLSSLATSNNSTVACIDRN--GLQSCPIKEDSFQRYSSDPTGAL-T
      |  :  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
RSSSTRSGGGDLTLGLEPSEEEAPRSP LAPSEGAGSDVFDGDLGMAAKGLOSLPTHDPSPIQRYSEDPTVPLPS

```

1070

E-D--SIDDTFLVP EYINQ-SV PKRPAGSVQN P VYHN Q L N P A P S R D P H Y Q D P H S T A V G N P E Y L N T V Q P T C V N S  
| | | | : | | | : | | | : | | : |  
ETDGYVA PLTCS PQPEYVNQPDVRPQPSPREGPLPAARPA GATLERPKTLSPGKNGVVKDVF AFGGAVENPEYLL  
1070 1120 1137 1290

1150

TFDSTFLPVEYINQ-SVPKRPAGSVQNPPVYHNQPLNPAFSDRDPHYQDPHSTAVGNPEYLNVTVQPTCVNSTFDSP  
 TPQGTCSPPQPEYVNQDDVRPQPSPREGPLPAARPAGATLERPKTLPSPGKNGVVKDVFAGGAVENPEYLTPOQG  
 1300

1210

AAAP-QPHPPAFSPAFDNLYYWDQDPPERGAPPS-TFKG-TPTAENPEYLGLDVPV  
 :  
 AAWAQKGS HQ- I SL-- DNP DYQ-QDFFPK EAKPN G I FKGS T--AENAEYLR-VAPQSSEFIGA  
 1255

**FIG. 4A**

MELAAALCRWGGLLALLPPGAASTQVC<sup>57</sup>TGTDMLRHLYPASPETHLDMLRHLTQGCQVVGQGNLELTLYLPPTNASLS  
MIIMEAAWCRWGFLLALLPFGIAGTQVC<sup>60</sup>TGTDMLRHLYPASPETHLDMLRHLTQGCQVVGQGNLELTLYVPANASLS

FLQDIQEVGQYVLIAHNQVRQVP LQRLRI VRGTQLFEDNYALAVLDNGDPLNNTTPVT-GASPGGLRELQ LRSLT  
 :  
 FLQDIQEVGQYMLIAHNQVKRVPLQRLRI VRGTQLFEDKYALAVLDNRDPQDNVAASTPGRTP EGLRELQ LRSLT

EILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRGWGESSEDCQSLTRTVCA  
 :  
 EILKGGVLIRGNPQLCYQDMVLWKDVFRKNNQLAPVDIDTNRSRACPPCAPACKDNHCWGESPEDCQILTGTICT

236  
GGCARCKGRLPTDCCHEQCAAGCTGPKHSDCLACLFHNHSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCV  
296  
SSGCARCKGRLPTDCCHEQCAAGCTGPKHSDCLACLFHNHSGICELHCPALVTYNTDTFESMHNPEGRYTFGASCV  
300

TTTTCPPNYLSTEVGSC TLVCP PNNQ EVTAEDGT QRC EKCSK PCARVCYGL GMEHLRG ARAITSD NVQEF DGCCKIF  
360

TTACPPNYLSTDVGSC TLVCP LHNQ EVTAEDGT QRC EKCSK PCARVCYGL GMEHLR EVRAVTSANI QEFA GCKKIF  
356

416

GSLAFLPESFDGDPASNTAPLQPEQLQVFETLEETGYLYISAWPDSLPLDSVFNQLQVIRGRILHNGAYSLTLQ  
||||| ||||| | ||| ||||| ||||| ||||| : ||||| ||||| |||||  
GSLAFLPESFDGDPSSGIAPLRPEQLQVFETLEETGYLYISAWPDSLRLDSVFNQLRIIRGRILHDGAYSLTLQ

420

FIG. 4B

476  
GLGISWLGLRSLRELGLALIHNNTHLCFVHTVPWDQLFRNPHQALLHTANRPEDE-CVGEGLACHQLCARGHC  
|||||  
GLGIHSLGLRSLRELGLALIHNRNAHLCFVHTVPWDQLFRNPHQALLHSGNRPEEDLCVSSGLVCNSLCAHGHC  
480

535  
WPGGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNAHCLPCHPECQPONGSVTCFGPEADQCVACAHYKDPFF  
|||||  
WPGGPTQCVNCSHFRLRGQECVEECRVWKGLPREYVSDKRCLPCHPECQPONSSETCFGSEADQCAACAHYKDDSSS  
540  
595  
600

655  
CVARCPSGVKPDL SYMPIWKFPDEEGACQPCPINC THSCVDLDDKGC PAEQRASPLT SIIISAVVGILLVVVLGVV  
|||||  
CVARCPSGVKPDL SYMPIWKYPDEEGICQPCPINC THSCVDLDERGCPAEQRASPVTFIIATVEGVLLFLILVVV  
660

710  
FGILL KRR-QQKIRKYTMRRLLQETELVEPLTPSGAMPNQAMRILKETELRKVKVLGSGAFGTVYKGIWIPD  
:|||||  
VGILL KRRRQ-KIRKYTMRRLLQETELVEPLTPSGAMPNQAMRILKETELRKVKVLGSGAFGTVYKGIWIPD  
715

770  
GENVKIPVAIKVLRENTSPKANKEILDEAYVMAGVGSPPYVSRLLGICLTSTVQLVTQLMPYGCCLLDHVRENHRL  
775  
GENVKIPVAIKVLRENTSPKANKEILDEAYVMAGVGSPPYVSRLLGICLTSTVQLVTQIMPYGCCLLDHVREHRL

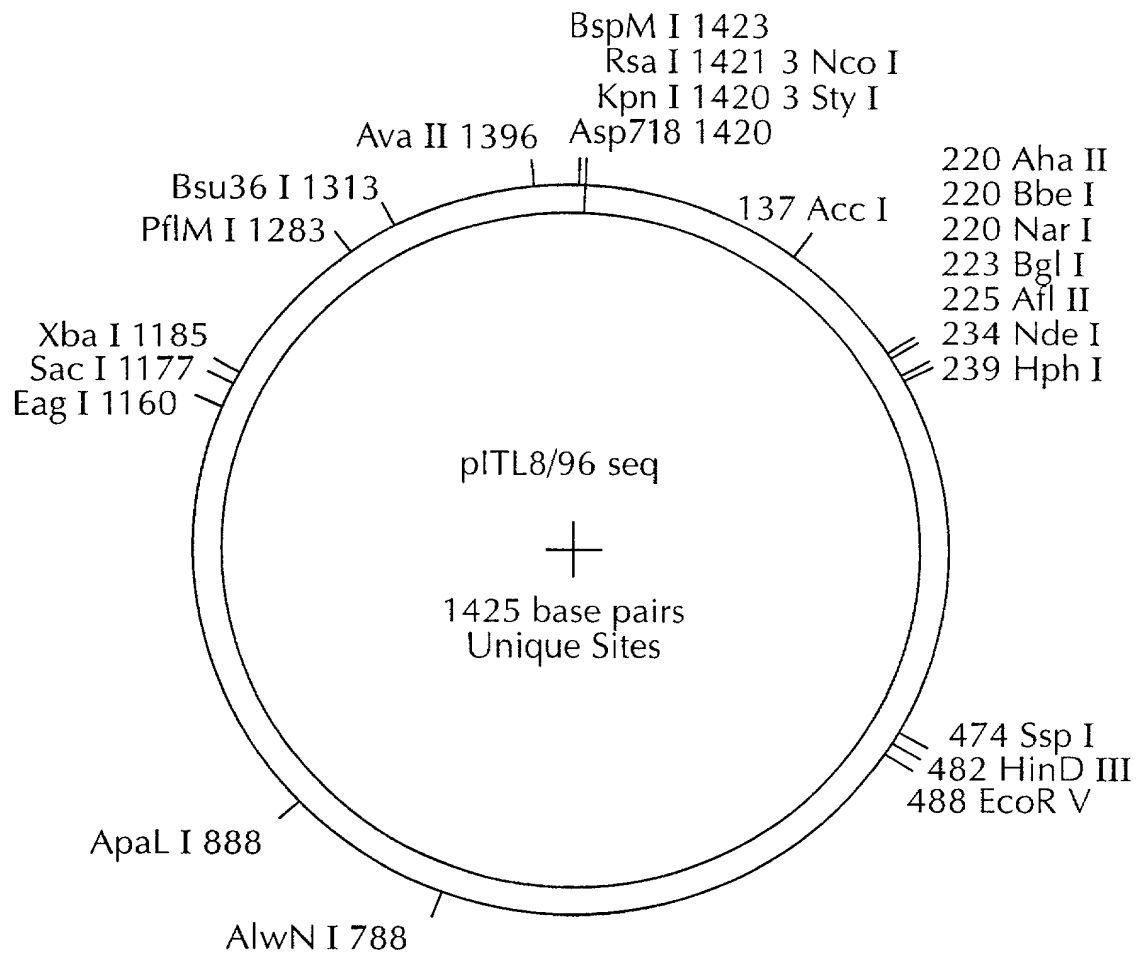
830  
GSQDLLNWCMIKAGMSYLEDVRLVHRDLAARNVLKSPNHVKITDFGLARLLDIDETEHADGGKVPKWMMALE  
835  
GSQDLLNWCMIKAGMSYLEDVRLVHRDLAARNVLKSPNHVKITDFGLARLLDIDETEHADGGKVPKWMMALE  
870  
896

FIG. 4C

SILRRRFTHQSDVWSYGVTVWELMTFGAKPYDGI PAREIPDLLEKGERLPQPPICTIDVYMIMVKCWMIDSECRP  
950  
SILRRRFTHQSDVWSYGVTVWEIMTFGAKPYDGI PAREIPDLLEKGERLPQPPICTIDVYMIMVKCWMIDSECRP  
955  
RFRELVSEFSRMARDPQRFVVIQNEEDLGPASPLDSTFYRSLLEDDDDMGDLVDAEEYLV PQQGFCDPAPGAGGM  
1010  
RFRELVSEFSRMARDPQRFVVIQNEEDLGPSSPMDSTFYRSLLEDDDDMGDLVDAEEYLV PQQGFSPDPTPGTGST  
1015  
VHHRHSSSTRSGGGDLTLGLEPSEEEAPRSLAPSEGAGSDVFDGDLGMGAAGLQSLPTHDPSP LQRYSEDPT  
1070  
AHRHSSSTRSGGGELTLGLEPSEEGPPRSLAPSEGAGSDVFDGDLAMGVTKGLQSLSPHDLSP LQRYSEDPT  
1075  
VPLPSETDGYVAPLTCSPQPEYVNQPDVVRPQPPSPREGPLPAARPA GATLERPKT LSPGKNGVVKDVFAFGGAVE  
1130  
LPLPPETDGYVAPLACSPQPEYVNQSEVQPPLTPEGPLPPVRPA GATLERPKT LSPGKNGVVKDVFAFGGAVE  
1135  
NPEYLT'PQGGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLG LDVPV  
1255  
NPEYLVPREGTASPPHPSPA FSPAFDNLYYWDQNSSEQGPPPSNFEGTPTAENPEYLG LDVPV  
1263



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**FIG. 5**

**COMBINED DECLARATION AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, the information given herein is true, that I believe I am the original, first and sole (if only one name is listed below) or an or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

A VECTOR FOR POLYNUCLEOTIDE VACCINES

which is described in: [x] PCT International Application No. PCT/US97/14306 filed August 14, 1997,  
which is U.S. application Serial No. 09/242,202 filed February 11, 1999.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known to me which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56 (a).

I hereby claim foreign priority benefits under Title 35 United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign applications(s) for patent or inventor's certificate or any PCT international applications(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

COUNTRY	APPLICATION	DATE OF FILING (DAY, MONTH, YEAR)	PRIORITY CLAIMED UNDER 35 USC § 119
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States Provisional patent application(s).

Provisional Application Serial No.	Filing Date	Status: patented, pending, abandoned
60/023,931	August 14, 1996	abandoned

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s) or PCT International application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

	Filing Date	Status: patented, pending, abandoned

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

James C. Haight, Reg. No. 25,588; Robert Benson, Reg. No. 33,612; Jack Spiegel, Reg. No. 34,477; Susan S. Rucker, Reg. No. 35,762; David R. Sadowski, Reg. No. 32,808; Steven M. Ferguson, Reg. No. 38,448; Stephen L. Finley, Reg. No. 36,357; John P. Kim, Reg. No. 38,514 and Joseph K. Hemby, Jr., Reg. No. 42,652, all of the Office of Technology Transfer, National Institutes of Health, 6011 Executive Boulevard, Suite 325, Rockville, Maryland 20852.

I further direct that all correspondence concerning this application be directed to:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of first joint inventor: Edward L. Nelson

Inventor's signature: 


Date: 10/15/99

Country of Citizenship: United States of America

Residence: 660 Buckhorn Road, Eldersburg, Maryland 21784 U.S.A. MD

Post Office Address: \_\_\_\_\_

Full Name of second joint inventor: Peter J. Nelson

Inventor's signature: 

Date: 10/21/99

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Residence: Reutterstrasse 70, D-80689 Munich, Germany DE X

Post Office Address: \_\_\_\_\_

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

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(A) NAME: THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES

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(F) POSTAL CODE: 20852

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(F) POSTAL CODE: 21784

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(F) POSTAL CODE: 80689

(ii) TITLE OF INVENTION: NOVEL VECTOR FOR POLYNUCLEOTIDE VACCINES

(iii) NUMBER OF SEQUENCES: 29

## (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: MORGAN & FINNEGAN, L.L.P.

(B) STREET: 345 PARK AVENUE

(C) CITY: NEW YORK

(D) STATE: NEW YORK

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(F) ZIP: 10154

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 MB

(B) COMPUTER: IBM PC COMPATIBLE

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: WORDPERFECT 5.1

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- (vi) CURRENT APPLICATION DATA:  
(A) FILING DATE: 14-AUG-1997
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: US60/023931  
(B) FILING DATE: 14-AUG-1996
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: KATHRYN M. BROWN  
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(C) REFERENCE/DOCKET NUMBER: 2026-4236PC
- (ix) TELECOMMUNICATION INFORMATION:  
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(C) TELEX: 421792

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 453 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:1:

GGCCGCGTTG	CTGGCGTTTT	TCCATAGGCT	CCGCCCCCCT	40
GACGAGCATC	ACAAAAATCG	ACGCTCAAGT	CAGAGGTGGC	80
GAAACCCGAC	AGGACTATAA	AGATACCAGG	CGTTTCCCCC	120
TGGAAGCTCC	CTCGTGCGCT	CTCCTGTTCC	GACCCTGCCG	160
CTTACCGGAT	ACCTCTCCGC	CTTTCTCCCT	TCGGGAAGCG	200
TGGCGCTTTC	TCAATGCTCA	CGCIGTAGGT	ATCTCAGTTC	240
GGTGTAGGTC	GTTCGCTCCA	AGCTGGGCTG	TGTGCACGAA	280
CCCCCGTIC	AGCCCGACCG	CTGCGCCTTA	TCCGGTAACT	320
ATCGTCTTGA	GTCCAACCCG	GTAAGACACG	ACTTATCGCC	360
ACTGGCAGCA	GCCACTGGTA	ACAGGATTAG	CAGAGCGAGG	400
TATGTAGGCG	GTGCTACAGA	GTTCTTGAAG	TGGTGGCCTA	440
ACTACGGCTA	CAC			453

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 453 base pairs  
(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:2:

GTGTAGCCGT	AGTTAGGCCA	CCACTTCAAG	AACTCTGTAG	40
CACCGCCTAC	ATACCTCGCT	CTGCTAATCC	TGTTACCAGT	80
GGCTGCTGCC	AGTGGCGATA	AGTCGTGTCT	TACCGGGTTG	120
GACTCAAGAC	GATAGTTACC	GGATAAGGCG	CAGCGGTCGG	160
GCTGAACGGG	GGGTTCGTGC	ACACAGCCCA	GCTTGGAGCG	200
AACGACCTAC	ACCGAACTGA	GATACCTACA	CCGTGAGCAT	240
TGAGAAAGCG	CCACGCTTCC	CGAAGGGAGA	AAGGCGGACA	280
GGTATCCGGT	AAGCGGCAGG	GTCGGAACAG	GAGAGCGCAC	320
GAGGGAGCTT	CCAGGGGGAA	ACGCCTGGTA	TCTTTATAGT	360
CCTGTCGGGT	TTCGCCACCT	CTGACTTGAG	CGTCGATTTT	400
TGTGATGCTC	GTCAGGGGGG	CGGAGCCTAT	GGAAAAACGC	440
CAGCAACGCG	GCC			453

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 210 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:3:

GAATTCTTTC	GGACTTTTGA	AAGTGATGGT	GGTGGCCGAA	40
GGATTCGAAC	CTTCGAAGTC	GATGACGGCA	GATTTAGAGT	80
CTGCTCCCTT	TGGCCGCTCG	GGAACCCAC	CACGGGTAAT	120
GCTTTTACTG	GCCTGCTCCC	TTATCGGGAA	GCGGGGCGCA	160
TCATATCAAA	TGACGCGCCG	CTGTAAAGTG	TTACGTTGAG	200
AAAGAATTC				210

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 209 base pairs

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(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:4:

GAATTCCTTTC	TCAACGTAAC	ACTTTACAGC	GGCGCGTCAT	40
TTGATATGAT	GCGCCCCGCT	TCCCGATAAG	GGAGCAGGCC	80
AGTAAAAGCA	TTACCCGTGG	TGGGGTTCCC	GAGCGGCCAA	120
AGGGAGCAGA	CTCTAAATCT	GCCGTCATCG	ACTTCGAAGG	160
TTCGAATCCT	TCCCCACCA	CCATCACTTT	CAAAAGTCCG	200
AAAGAATTC				209

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 6 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:

AATAAA

6

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 6 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:6:

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ATTAAA

6

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:7:

AGTAAA

6

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:8:

AAGAAC

6

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No



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(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:9:

AATACA

6

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 228 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:10:

GCCTTAAGGG	CCATATGGTG	AGTGGATCCC	TTGACCCCAG	40
GCGGGGATGG	GGAGACCTG	TAGTCAGAGC	CCCCGGGCAG	80
CACAGGCCAA	TGCCCCGTCCT	TCCCCTGCAG	GATGAGTAGT	120
GAGTGCCTCT	CCTGGCCCTG	GAAGTTGCCA	CTCCAGTGCC	160
CACCAGCCTT	GTCCTAATAA	AATTAAGTTG	CATCATTTTG	200
TCTGACTAGG	TGTCCTCTAT	AATATTAT		228

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 228 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:11:

ATAATATTAT	AGAGGACACC	TAGTCAGAAC	AAATGATGCA	40
ACTTAATTTT	ATTAGGACAA	GGCTGGTGGG	CACTGGAGTG	80
GCAACTTCCA	GGGCCAGGAG	AGGCACTCAC	TACTCATCCT	120

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GCAGGGGAAG	GACGGGCATT	GGCCTGTGCT	GCCCGGGGGC	160
TCTGACTACA	GGTCTCCCCC	ATCCCCGCCT	GGGGTCAAGG	200
CATCCACTCA	CCATATGGCC	CTTAAGG		228

## (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 252 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:12:

CCTCGGTACC	TGCCATGGCG	CGGATTCTTT	ATCACTGATA	40
AGTTGGTGGA	CATATTATGT	TTATCAGTGA	TAAAGTGTCA	80
AGCATGACAA	AGTTGCAGCC	GAATACAGTG	ATCCGTGCCG	120
GCCCTGGACT	GTTGAACGAG	GTCGGCGTAG	ACGGTCTGAC	160
GACACGCAAA	CTGGCGGAAC	GGTTGGGGGT	GCAGCAGCCG	200
GCGCTTTACT	GGCACTTCAG	GAACAAGCGG	GCGCCTTAAG	240
GGCCATATGC	CG			252

## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 35 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No

## (xi) SEQUENCE DESCRIPTION:SEQ ID NO:13:

CCTCGGTACC	TGCCACCATG	GCGCGGATTC	TTTAT	35
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## (2) INFORMATION FOR SEQ ID NO:14:

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- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 38 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:14:

CGGCATATGG CCTTAAGGCG CCCGCTTGTT CCTGAAGT

38

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 228 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:15:

GCCTTAAGGG CCATATGGTG AGTGGATGCC TTGACCCCAG	40
GCGGGGATGG GGGAGACCTG TAGTCAGAGC CCCCGGGCAG	80
CACAGGCCAA TGCCCGTCCT TCCCCTGCAG GATGAGTAGT	120
GAGTGCCTCT CCTGGCCCTG GAAGTTGCCA CTCCAGTGCC	160
CACCAGCCTT GTCCTAATAA AATTAAGTTG CATCATTTTG	200
TCTGACTAGG TGTCCTCTAT AATATTAT	228

## (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1425 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA

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- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:16:

TGCCATGGCG	CGGATTCTTT	ATCACTGATA	AGTTGGTGGA	40
CATATTATGT	TTATCAGTGA	TAAAGTGTCA	AGCATGACAA	80
AGTTGCAGCC	GAATACAGTG	ATCCGTGCCG	GCCCTGGACT	120
GTTGAACGAG	GTCGGCGTAG	ACGGTCTGAC	GACACGCAAA	160
CTGGCGGAAC	GGTTGGGGGT	GCAGCAGCCG	GCGCTTTACT	200
GGCACTTCAG	GAACAAGCGG	GCGCCTTAAG	GGCCATATGG	240
TGAGTGGATG	CCTTGACCCC	AGGCGGGGAT	GGGGGAGACC	280
TGTAGTCAGA	GCCCCCGGGC	AGCACAGGCC	AATGCCCCGTC	320
CTTCCCCCTGC	AGTGAGTAGT	GA CTGCCCCG	GTGGGATCCC	360
TGTGACCCCT	CCCCAGTGCC	TCTCCTGGCC	CTGGAAGTTG	400
CCACTCCAGT	GCCCACCAGC	CTTGTCCTAA	TAAAATTAAG	440
TTGCATCATT	TTGTCTGACT	AGGTGTCTCT	TATAATATTA	480
TAAGCTTGAT	ATCGAATTCT	TTCTCAACGT	AACACTTTAC	520
AGCGGCGCGT	CATTTGATAT	GATGCGCCCC	GCTTCCCCGAT	560
AAGGGAGCAG	GCCAGTAAAA	GCATTACCCG	TGGTGGGGTT	600
CCCGAGCGGC	CAAAGGGAGC	AGACTCTAAA	TCTGCCGTCA	640
TCGACTTCGA	AGGTTCGAAT	CCTTCCCCCA	CCACCATCAC	680
TTTCAAAAGT	CCGAAAGAAT	TCCTGCAGCC	CGTGTAGCCG	720
TAGTTAGGCC	ACCACCTCAA	GA ACTCTGTA	GCACCGCCTA	760
CATACCTCGC	TCTGCTAATC	CTGTTACCAG	TGGCTGCTGC	800
CAGTGGCGAT	AAGTCGTGTC	TTACCGGGTT	GGACTCAAGA	840
CGATAGTTAC	CGGATAAGGC	GCAGCGGTCG	GGCTGAACGG	880
GGGGTTCGTG	CACACAGCCC	AGCTTGAGAC	GAACGACCTA	920
CACCGAACTG	AGATACCTAC	AGCGTGAGCA	TTGAGAAAGC	960
GCCACGCTTC	CCGAAGGGAG	AAAGGCGGAC	AGGTATCCGG	1000
TAAGCGGCAG	GGTCGGAACA	GGAGAGCGCA	CGAGGGAGCT	1040
TCCAGGGGGA	AACGCCTGGT	ATCTTTATAG	TCCTGTCGGG	1080
TTTCGCCACC	TCTGACTTGA	GCGTCGATTT	TTGTGATGCT	1120
CGTCAGGGGG	GCGGAGCCTA	TGGAAAAACG	CCAGCAACGC	1160
GGCCGGGGGA	TCCGGAGAGC	TCACTCTAGA	TGAGAGAGCA	1200
GTGAGGGAGA	GACAGAGACT	CGAATTTCCG	GAGCTATTTT	1240
AGTTTTCTTT	TCCGTTTTGT	GCAATTTTAC	TTATGATACC	1280
GGCCAATGCT	TGGTTGCTAT	TTTGAAACT	CCCCTTAGGG	1320
GATGCCCTC	AACTGGCCCT	ATAAAGGGCC	AGCCTGAGCT	1360
GCAGAGGATT	CCTGCAGAGG	ATCAAGACAG	CACGTGGACC	1400
TCGCACAGCC	TCTCCACAG	GTACC		1425

## (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 720 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

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- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:17:

ATGAGCAAG	GGCGAGGAAC	TGTTCACTGG	CGTGGTCCCA	40
ATTCTCGTGG	AACTGGATGG	CGATGTGAAT	GGGCACAAAT	80
TTTCTGTCAG	CGGAGAGGGT	GAAGGTGATG	CCACATACGG	120
AAAGCTCACC	CTGAAATTCA	TCTGCACCAC	TGGAAAGCTC	160
CCTGTGCCAT	GGCCAACACT	GGTCACTACC	TTCACCTATG	200
GCGTGCACTG	CTTTTCCAGA	TACCCAGACC	ATATGAACGA	240
GCATGACTTT	TTCAAGAGCG	CCATGCCCGA	GGGCTATGTG	280
CAGGAGAGAA	CCATCTTTTT	CAAAGATGAC	GGGAAC TACA	320
AGACCCGCGC	TGAAGTCAAG	TTCGAAGGTG	ACACCCTGGT	360
GAATAGAATC	GAGTTGAAGG	GCATTGACTT	TAAGGAAGAT	400
GGAAACATTC	TCGGCCACAA	GCTGGAATAC	AACTATAACT	440
CCCACAATGT	GTACATCATG	GCCGACAAGC	AAAAGAATGG	480
CATCAAGGTC	AACTTCAAGA	TCAGACACAA	CATTGAGGAT	520
GGATCCGTGC	AGCTGGCCGA	CCATTATCAA	CAGAACACTC	560
CAATCGGCCA	CCGCCCTGTG	CTCCTCCCAG	ACAACAATTA	600
CCTGTCCACC	CAGTCTGCCC	TGTCTAAAGA	TCCCAACGAA	640
AAGAGAGACC	ACATGGTCCT	GCTGGAGTTT	GTGACCGCTG	680
CTGGGATCAC	ACATGGCATG	GACGAGCTGT	ACAAGTGAGC	720

## (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1911 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:18:

TATGAGCAAG	GGCGAGGAAC	TGTTCACTGG	CGTGGTCCCA	40
ATTCTCGTGG	AACTGGATGG	CGATGTGAAT	GGGCACAAAT	80
TTTCTGTCAG	CGGAGAGGGT	GAAGGTGATG	CCACATACGG	120
AAAGCTCACC	CTGAAATTCA	TCTGCACCAC	TGGAAAGCTC	160
CCTGTGCCAT	GGCCAACACT	GGTCACTACC	TTCACCTATG	200
GCGTGCACTG	CTTTTCCAGA	TACCCAGACC	ATATGAAGCA	240
GCATGACTTT	TTCAAGAGCG	CCATGCCCGA	GGGCTATGTG	280

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CAGGAGAGAA	CCATCTTTTT	CAAAGATGAC	GGGAACTACA	320
AGACCCGCGC	TGAAGTCAAG	TTCGAAGGTG	ACACCCTGGT	360
GAATAGAATC	GAGTTGAAGG	GCATTGACTT	TAAGGAAGAT	400
GGAAACATTC	TCGGCCACAA	GCTGGAATAC	AACTATAACT	440
CCCACAATGT	GTACATCATG	GCCGACAAGC	AAAAGAATGG	480
CATCAAGGTC	AACTTCAAGA	TCAGACACAA	CATTGAGGAT	520
GGATCCGTGC	AGCTGGCCGA	CCATTATCAA	CAGAACACTC	560
CAATCGGCGA	CGGCCCTGTG	CTCCTCCCAG	ACAACCATTA	600
CCTGTCCACC	CAGTCTGCCC	GTCTAAAGAT	CCCAACGAAA	640
AGAGAGACCA	CATGGTCCTG	CTGGAGTTTG	TGACCGCTGC	680
TGGGATCACA	CATGGCATGG	ACGAGCTGTA	CAAGTGAGCC	720
ATATGGTGAG	TGGATGCCTT	GACCCAGGC	GGGGATGGGG	760
GAGACCTGTA	GTCAGAGCCC	CCGGGCAGCA	CAGGCCAATG	800
CCCGTCCTTC	CCCTGCAGTG	AGTAGTGACT	GCCCGGGTGG	840
GATCCCTGTG	ACCCCTCCCC	AGTGCCTCTC	CTGGCCCTGG	880
AAGTTGCCAC	TCCAGTGCCC	ACCAGCCTTG	TCCTAATAAA	920
ATTAAGTTGC	ATCATTTTGT	CTGACTAGGT	GTCCCTCTATA	960
ATATTATAAG	CTTGATATCG	AATTCTTTCT	CAACGTAACA	1000
CTTTACAGCG	GCGCGTCATT	TGATATGATG	CGCCCCGCTT	1040
CCCGATAAGG	GAGCAGGCCA	GTAAAAGCAT	TACCCGTGGT	1080
GGGGTTCCCC	AGCGGCCAAA	GGGAGCAGAC	TCTAAATCTG	1120
CCGTCATCGA	CTTCGAAGGT	TCGAATCCTT	CCCCCACCAC	1160
CATCACTTTC	AAAAGTCCGA	AAGAATTCCT	GCAGCCCGTG	1200
TAGCCGTAGT	TAGGCCACCA	CTTCAAGAAC	TCTGTAGCAC	1240
CGCCTACATA	CCTCGCTCTG	CTAATCCTGT	TACCAGTGGC	1280
TGCTGCCAGT	GGCGATAAGT	CGTGTCTTAC	CGGGTTGGAC	1320
TCAAGACGAT	AGTTACCGGA	TAAGGCGCAG	CGGTCGGGCT	1360
GAACGGGGGG	TTCGTGCACA	CAGCCCAGCT	TGGAGCGAAC	1400
GACCTACACC	GAAGTGAGAT	ACCTACAGCG	TGAGCATTGA	1440
GAAAGCGCCA	CGCTTCCCGA	AGGGAGAAAG	GCGGACAGGT	1480
ATCCGGTAAG	CGGCAGGGTC	GGAACAGGAG	AGCGCACGAG	1520
GGAGCTTCCA	GGGGGAAACG	CCTGGTATCT	TTATAGTCCT	1560
GTCGGGTTTC	GCCACCTCTG	ACTTGAGCGT	CGATTTTTGT	1600
GATGCTCGTC	AGGGGGGCGG	AGCCTATGGA	AAAACGCCAG	1640
CAACGCGGCC	GGGGGATCCG	GAGAGCTCAC	TCTAGATGAG	1680
AGAGCAGTGA	GGGAGAGACA	GAGACTCGAA	TTTCCGGAGC	1720
TATTTCAAGT	TTCTTTTCCG	TTTGTGCAA	TTTCACTTAT	1760
GATACCGGCC	AATGCTTGGT	TGCTATTTTG	GAAACTCCCC	1800
TTAGGGGATG	CCCCTCAACT	GGCCCTATAA	AGGGCCAGCC	1840
TGAGCTGCAG	AGGATTCCTG	CAGAGGATCA	AGACAGCACG	1880
TGGACCTCGC	ACAGCCTCTC	CCACAGGTAC	C	1911

## (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 69 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

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Pro Asp Leu Ser Tyr Met Pro Ile Trp Lys Phe Pro
 1      5      10
Asp Glu Glu Gly Ala Cys Gln Pro Cys Pro Ile Asn
      15      20
Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys Gly
25      30      35
Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser
      40      45
Ile Ile Ser Ala Val Val Gly Ile Leu Leu Val Val
50      55      60
Val Leu Gly Val Val Phe Gly Ile Leu
      65

```

## (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 287 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:20:

```

Pro Ala Pro Gly Ala Gly Gly Met Val His His Arg
 1      5      10
His Arg Ser Ser Ser Thr Arg Ser Gly Gly Gly Asp
      15      20
Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu Ala
25      30      35
Pro Arg Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly
      40      45
Ser Asp Val Phe Asp Gly Asp Leu Gly Met Gly Ala
50      55      60
Ala Lys Gly Leu Ser Leu Pro Thr His Asp Pro Ser
      65      70
Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Val Pro
      75      80
Leu Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu
85      90      95
Thr Cys Ser Pro Gln Pro Glu Tyr Val Asn Gln Pro
      100      105
Asp Val Arg Pro Pro Pro Ser Pro Arg Glu Gly Pro
110      115      120
Leu Pro Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu
      125      130
Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val
135      140

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Val Lys Asp Val Phe Ala Phe Gly Gly Ala Val Glu  
 145 150 155  
 Asn Pro Glu Tyr Leu Thr Pro Gln Gly Thr Cys Ser  
 160 165  
 Pro Gln Pro Glu Tyr Val Asn Gln Pro Asp Val Arg  
 170 175 180  
 Pro Gln Pro Pro Ser Pro Arg Glu Gly Pro Leu Pro  
 185 190  
 Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu Arg Pro  
 195 200  
 Lys Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp  
 205 210 215  
 Val Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu  
 220 225  
 Tyr Leu Thr Pro Gln Gly Gly Ala Ala Pro Gln Pro  
 230 235 240  
 His Pro Pro Pro Ala Phe Ser Pro Ala Phe Asp Asn  
 245 250  
 Leu Tyr Tyr Trp Asp Asp Pro Pro Glu Arg Gly Ala  
 255 260  
 Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala Glu  
 260 270 275  
 Asn Pro Glu Tyr Leu Gly Leu Asp Val Pro Val  
 280 285

## (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 22 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:21:

Ile Ile Ser Ala Val Val Gly Ile Leu Leu Val Val  
 1 5 10  
 Val Leu Gly Val Val Phe Gly Ile Leu Ile  
 15 20

## (2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2145 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown



GCCACCATGG	CCCCTGACCT	CTCCTACATG	CCCATCTGGA	40
AGTTTCCAGA	TGAGGAGGGC	GCATGCCAGC	CTTGCCCCAT	80
CAACTGCACC	CACTCCTGTG	TGGACCTGGA	TGACAAGGGC	120
TGCCCCGCCG	AGCAGAGAGC	CAGCCCTCTG	ACGTCCATCA	160
TCTCTGCGGT	GGTTGGCATT	CTGCTGGTCG	TGGTCTTGGG	200
GGTGGTCTTT	GGGATCCTCA	TCAAGCGACG	GCAGCAGAAG	240
ATCACATGTC	CAGACCCTGC	CCCGGGCGCT	GGGGGCATGG	280
TCCACCACAG	GCACCGCAGC	TCATCTACCA	GGAGTGGCGG	320
TGGGGACCTG	ACACTAGGGC	TGGAGCCCTC	TGAAGAGGAG	360
GCCCCCAGGT	CTCCACTGGC	ACCCTCCGAA	GGGGCTGGCT	400
CCGATGTATT	TGATGGTGAC	CTGGGAATGG	GGGCAGCCAA	440
GGGGCTGCAA	AGCCTCCCCA	CACATGACCC	CAGCCCTCTA	480
CAGCGGTACA	GTGAGGACCC	CACAGTACCC	CTGCCCTCTG	520
AGACTGATGG	CTACGTTGCC	CCCCTGACCT	GCAGCCCCCA	560
GCCTGAATAT	GTGAACCAGC	CAGATGTTTC	GCCCCAGCCC	600
CCTTCGCCCC	GAGAGGGCCC	TCTGCCTGCT	GCCCCGACCTG	640
CTGGTGCCAC	TCTGGAAAGG	CCCAAGACTC	TCTCCCCAGG	680
GAAGAATGGG	GTCGTCAAAG	ACGTTTTTTC	CTTTGGGGGT	720
GCCGTGGAGA	ACCCCGAGAC	TTGACACCCC	AGGGAGGAGC	760
TGCCCCCTCAG	CCCCACCCTC	CTCCTGCCTT	CAGCCCAGCC	800
TTCGACAACC	TCTATTACTG	GGACCAGGAC	CCACCAGAGC	840
GGGGGGCTCC	ACCCAGCACC	TTCAAAGGGA	CACCTACGGC	880
AGAGAACCCA	GAGTACCTGG	GTCTGGACGT	GCCAGTGTGA	920
AGCCTTAAGG	GCCATATGGT	GAGTGGATGC	CTTGACCCCCA	960
GGCGGGGATG	GGGGAGACCT	GTAGTCAGAG	CCCCCGGGCA	1000
GCACAGGCCA	ATGCCCGTCC	TTCCCCTGCA	GTCGAGTAGT	1040
ACTGCCCCGG	TGGGATCCCT	GTGACCCCTC	CCCAGTGCCT	1080
CTCCTGGCCC	TGGAAGTTGC	CACTCCAGTG	CCCACCAGCC	1120
TTGTCTTAAT	AAAATTAAGT	TGCATCATTT	TGTCTGACTA	1160
GGTGTCTCT	ATAATATTAT	AAGCTTGATA	TCGAATTCTT	1200
TCTCAACGTA	ACACTTTACA	GCGGCGCGTC	ATTTGATATG	1240
ATGCGCCCCG	CTTCCCGATA	AGGGAGCAGG	CCAGTAAAAG	1280
CATTACCCGT	GGTGGGGTTC	CCGAGCGGCC	AAAGGGAGCA	1320
GACTCTAAAT	CTGCCGTCAT	CGACTTCGAA	GGTTCGAATC	1360
CTTCCCCCAC	CACCATCACT	TTCAAAAGTC	CGAAAGAATT	1400
CCTGCAGCCC	GTGTAGCCGT	AGTTAGGCCA	CCACTTCAAG	1440
AACTCTGTAG	CACCGCCTAC	ATACCTCGCT	CTGCTAATCC	1480
TGTTACCAGT	GGCTGCTGCC	AGTGGCGATA	AGTCGTGTCT	1520
TACCGGGTTG	GACTCAAGAC	GATAGTTACC	GGATAAGGCG	1560
CAGCGGTCGG	GCTGAACGGG	GGGTTCTGTC	ACACAGCCCA	1600
GCTTGAGCG	AACGACCTAC	ACCGAACTGA	GATACCTACA	1640
GCGTGAGCAT	TGAGAAAGCG	CCACGCTTCC	CGAAGGGAGA	1680
AAGGCGGACA	GGTATCCGGT	AAGCGGCAGG	GTCGGAACAG	1720
GAGAGCGCAC	GAGGGAGCTT	CCAGGGGGAA	ACGCCTGGTA	1760

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TCTTTATAGT	CCTGTCGGGT	TTCGCCACCT	CTGACTTGAG	1820
CGTCGATTTT	TGTGATGCTC	GTCAGGGGGG	CGGAGCCTAT	1860
GGAAAAACGC	CAGCAACGCG	GCCGGGGGAT	CCGGAGAGCT	1900
CACTCTAGAT	GAGAGAGCAG	TGAGGGAGAG	ACAGAGACTC	1940
GAATTTCCGG	AGCTATTTCA	GTTTTCTTTT	CCGTTTTGTG	1980
CAATTTCACT	TATGATACCG	GCCAATGCTT	GGTTGCTATT	2020
TTGGAAACTC	CCCTTAGGGG	ATGCCCCTCA	ACTGGCCCTA	2060
TAAAGGGCCA	GCCTGAGCTG	CAGAGGATTC	CTGCAGAGGA	2100
TCAAGACAGC	ACGTGGACCT	CGCACAGCCT	CTCCCACAGG	2140
TACCT				2145

## (2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: oligonucleotide
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:23:

GTCTGCCACC ATGGCCTACT CCCCTGC

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## (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: oligonucleotide
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:24:

TTCTTTGGTG ACCTACCTCT TCGGAATTGC CGAGTC

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## (2) INFORMATION FOR SEQ ID NO:25:

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- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1242 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATGGAGGAGC	CGCAGTCAGA	TCCTAGCGTC	GAGCCCCCTC	40
TGAGTCAGGA	AACATTTTCA	GACCTATGGA	AACTACTTCC	80
TGAAAACAAC	GTTCTGTCCC	CCTTGCCGTC	CCAAGCAATG	120
GATGATTTGA	TGCTGTCCCC	GGACGATATT	GAACAATGGT	160
TCACTGAAGA	CCCAGGTCCA	GATGAAGCTC	CCAGAATGCC	200
AGAGGCTGCT	CCCCGCGTGG	CCCCTGCACC	AGCAGCTCCT	240
ACACCGGCGG	CCCCTGCACC	AGCCCCCTCC	TGGCCCCCTGT	280
CATCTTCTGT	CCCTTCCCAG	AAAACCTACC	AGGGCAGCTA	320
CGGTTTCCGT	CTGGGCTTCT	TGCATTCTGG	GACAGCCAAG	360
TCTGCCACCA	TGGCCTACTC	CCCTGCGTCT	GTGACTTGCA	400
CGTACTCCCC	TGCCCTCAAC	AAGATGTTTT	GCCAACTGGC	440
CAAGACCTGC	CCTGTGCAGC	TGTGGGTTGA	TTCCACACCC	480
CCGCCCCGCA	CCCGCGTCCG	CGCCATGGCC	ATCTACAAGC	520
AGTCACAGCA	CATGACGGAG	GTTGTGAGGC	GCTGCCCCCA	560
CCATGAGCGC	TGCTCAGATA	GCGATGGTCT	GGCCCCCTCCT	600
CAGCGTCTTA	TCCGAGTGGA	AGGAAATTTG	CGTGTGGAGT	640
ATTTGGATGA	CAGAAACACT	TTTCGACATA	GTGTGGTGGT	680
GCCCTATGAG	CCGCCTGAGG	TTGGCTCTGA	CTGTACCACC	720
ATCCACTACA	ACTACATGTG	TAACAGTTCC	TGCATGGGCG	760
GCATGAACCG	GAGGCCCATC	CTCACCATCA	TCACACTGGA	800
AGACTCCAGT	GGTAATCTAC	TGGGACGGAA	CAGCTTTGAG	840
GTGCGTGTTT	GTGCCTGTCC	TGGGAGAGAC	CGGCGCACAG	880
AGGAAGAGAA	TCTCCGCAAG	AAAGGGGAGC	CTCACCACGA	920
GCTGCCCCCA	GGGAGCACTA	AGCGAGCACT	GCCCAACAAC	960
ACCAGCTCCT	CTCCCCAGCC	AAAGAAGAAA	CCACTGGATG	1000
GAGAATATTT	CACCCTTCAG	ATCCGTGGGC	GTGAGCGCTT	1040
CGAGATGTTT	TTTGGTGACC	TACCTCTTCG	GAATTGCCGA	1080
GTCTTCCGAG	AGCTGAATGA	GGCCTTGGA	CTCAAGGATG	1120
CCCAGGCTGG	GAAGGAGCCA	GGGGGGAGCA	GGGCTCACTC	1160
CAGCCACCTG	AAGTCCAAAA	AGGGTCAGTC	TACCTCCCGC	1200
CATAAAAAAC	TCATGTTCAA	GACAGAAGGG	CCTGACTCAG	1240
AC				1242

(2) INFORMATION FOR SEQ ID NO:26:

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- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 608 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:26:

CTCGGGCCGC	GTTGCTGGCG	TTTTTCCATA	GGCTCCGCCC	40
CCCTGACGAG	CATCACAAAA	ATCGACGCTC	AAGTCAGAGG	80
TGGCGAAACC	CGACAGGACT	ATAAAGATAC	CAGGCGTTTC	120
CCCCTGGAAG	CTCCCTCGTG	CGCTCTCCTG	TTCCGACCCT	160
GCCGCTTACC	GGATACCTGT	CCGCCTTTCT	CCCTTCGGGA	200
AGCGTGGCGC	TTTCTCAATG	CTCACGCTGT	AGGTATCTCA	240
GTTCGGTGTA	GGTCGTTTCG	TCCAAGCTGG	GCTGTGTGCA	280
CGAACCCCCC	G TTCAGCCCG	ACCGCTGCGC	CTTATCCGGT	320
AACTATCGTC	TTGAGTCCAA	CCCGGTAAGA	CACGACTTAT	360
CGCCACTGGC	AGCAGCCACT	GGTAACAGGA	TTAGCAGAGC	400
GAGGTATGTA	GGCGGTGCTA	CAGAGTTCTT	GAAGTGGTGG	440
CCTAACTACG	GCTACACTAG	AAGGACAGTA	TTTGGTATCT	480
GCGCTCTGCT	GAAGCCAGTT	ACCTTCGGAA	AAAGAGTTGG	520
TAGCTCTTGA	TCCGGCAAAC	AAACCACCGC	TGGTAGCGGT	560
GGTTTTTTTG	TTTGCAAGCA	GCAGATTACG	CGCAGAAAAA	600
AAGGATCT				608

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1547 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:27:

GGTACCTGCC	ACCATGGCGC	GGATTCTTTA	TCACTGATAA	40
GTTGGTGGAC	ATATTATGTT	TATCAGTGAT	AAAGTGTCAA	80
GCATGACAAA	GTTGCAGCCG	AATACAGTGA	TCCGTGCCCG	120

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CCCTGGACTG	TTGAACGAGG	TCGGCGTAGA	CGGTCTGACG	160
ACACGCAAAC	TGGCGGAACG	GTTGGGGGTG	CAGCAGCCGG	200
CGCTTTACTG	GCACTTCAGG	AACAAGCGGG	CGCCTTAAGG	240
GCCATATGGT	GAGTGGATGC	CTTGACCCCA	GGCGGGGATG	280
GGGGAGACCT	GTAGTCAGAG	CCCCCGGGCA	GCACAGGCCA	320
ATGCCCCGTC	TTCCCCTGCA	GGATGAGTAG	TGAGTGCCTC	360
TCCTGGCCCT	GGAAGTTGCC	ACTCCAGTGC	CCACCAGCCT	400
TGTCCTAATA	AAATTAAGTT	GCATCATTTT	GTCTGACTAG	440
GTGTCCTCTA	TAATATTATA	AGCTTGATAT	CGAATTCTTT	480
CGGACTTTTG	AAAGTGATGG	TGGTGGGGGA	AGGATTTCGAA	520
CCTTCGAAGT	CGATGACGGC	AGATTTAGAG	TCTGCTCCCT	560
TTGGCCGCTC	GGGAACCCCA	CCACGGGTAA	TGCTTTTACT	600
GGCCTGCTCC	CTTATCGGGA	AGCGGGGCGC	ATCATATCAA	640
ATGACGCGCC	GCTGTAAAGT	GTTACGTTGA	GAAAGAATTC	680
CTGCAGCCCG	CCGCGTTGCT	GGCGTTTTTC	CATAGGCTCC	720
GCCCCCCTGA	CGAGCATCAC	AAAAATCGAC	GCTCAAGTCA	760
GAGGTGGCGA	AACCCGACAG	GACTATAAAG	ATACCAGGCG	800
TTTCCCCCTG	GAAGCTCCCT	CGTGCGCTCT	CCTGTTCCGA	840
CCCTGCCGCT	TACCGGATAC	CTGTCCGCCT	TTCTCCCTTC	880
GGGAAGCGTG	GCGCTTTCTC	AATGCTCACG	CTGTAGGTAT	920
CTCAGTTCGG	TGTAGGTCGT	TCGCTCCAAG	CTGGGCTGTG	960
TGCACGAACC	CCCCGTTTCA	CCCGACCGCT	GCGCCTTATC	1000
CGGTAACAT	CGTCTTGAGT	CCAACCCGGT	AAGACACGAC	1040
TTATCGCCAC	TGGCAGCAGC	CACTGGTAAC	AGGATTAGCA	1080
GAGCGAGGTA	TGTAGGCGGT	GCTACAGAGT	TCTTGAAGTG	1120
GTGGCCTAAC	TACGGCTACA	CTAGAAGAGC	AGTATTTGGT	1160
ATCTGCGCTC	TGCTGAAGCC	AGTTACCTTC	GGAAAAAGAG	1200
TTGGTAGCTC	TTGATCCGGC	AAACAAACCA	CCGCTGGTAG	1240
CGGTGGTTTT	TTTGTTTGCA	AGCAGCAGAT	TACGCGCAGA	1280
AAAAAAGGAT	CTGGGGGATC	CGGAGAGCTC	ACTCTAGATG	1320
AGAGAGCAGT	GAGGGAGAGA	CAGAGACTCG	AATTTCCGGA	1360
GCTATTTTCA	TTTTCTTTTC	CGTTTTGTGC	AATTTCACTT	1400
ATGATACCGG	CCAATGCTTG	GTGCTATTTT	TGGAAACTCC	1440
CCTTAGGGGA	TGCCCCCTCA	CTGGCCCTAT	AAAGGGCCAG	1480
CCTGAGCTGC	AGAGGATTCC	TGCAGAGGAT	CAAGACAGCA	1520
CGTGGACCTC	GCACAGCCTC	TCCCACA		1547

## (2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1847 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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GGTACCTGCC ACCATGGCGC GGATTCTTTA TCACTGATAA 40  
 GTTGGTGGAC ATATTATGTT TATCAGTGAT AAAGTGTCAA 80  
 GCATGACAAA GTTGCAGCCG AATACAGTGA TCCGTGCCGG 120  
 CCCTGGACTG TTGAACGAGG TCGGCGTAGA CGGTCTGACG 160  
 ACACGCAAAC TGGCGGAACG GTTGGGGGTG CAGCAGCCGG 200  
 CGCTTTACTG GCACTTCAGG AACAAAGCGGG CGCCTTAAGG 240  
 GCCATATGGT GAGTGGATGC CTTGACCCCA GGCGGGGATG 280  
 GGGGAGACCT GTAGTCAGAG CCCCCGGGCA GCACAGGCCA 320  
 ATGCCCGTCC TTCCCCTGCA GGATGAGTAG TGAGTGCCTC 360  
 TCCTGGCCCT GGAAGTTGCC ACTCCAGTGC CCACCAGCCT 400  
 TGTCCCTAATA AAATTAAGTT GCATCATTTT GTCTGACTAG 440  
 GTGTCCTCTA TAATATTATA AGCTTGATAT CGAATTCTTT 480  
 CGGACTTTTG AAAGTGATGG TGGTGGGGGA AGGATTCGAA 520  
 CCTTCGAAGT CGATGACGGC AGATTTAGAG TCTGCTCCCT 560  
 TTGGCCGCTC GGGAAACCCA CCACGGGTAA TGCTTTTACT 600  
 GGCCTGCTCC CTTATCGGGA AGCGGGGCGC ATCATATCAA 640  
 ATGACGCGCC GCTGTAAAGT GTTACGTTGA GAAAGAATTC 680  
 CTGCAGCCCG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC 720  
 GCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA 760  
 GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG 800  
 TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA 840  
 CCCTGCCGCT TACCGGATAC CTGTCCGCTT TTCTCCCTTC 880  
 GGGAAGCGTG GCGCTTTCTC AATGCTCACG CTGTAGGTAT 920  
 CTCAGTTCGG TGTAGTTCGT TCGCTCCAAG CTGGGCTGTG 960  
 TGCACGAACC CCGCGTTCAG CCCGACCGCT GCGCCTTATC 1000  
 CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC 1040  
 TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA 1080  
 GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG 1120  
 GTGGCCTAAC TACGGCTACA CTAGAAGGAC AGTATTTGGT 1160  
 ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG 1200  
 TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG 1240  
 CCGTGTTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA 1280  
 AAAAAAGGAT CTGGGGGATC CGGAGAGCTC CCAACGCGTT 1320  
 GGATGCATGG ATGAGGGAAA GGAGGTAAGA TCTGTAATGA 1400  
 ATAAGCAGGA ACTTTGAAGA CTCAGTGACT CAGTGAGTAA 1440  
 TAAAGACTCA GTGACTTCTG ATCCTGTCTT AACTGCCACT 1480  
 CCTTGTTGTC CCAAGAAAGC GGCTTCCTGC TCTCTGAGGA 1520  
 GGACCCCTTC CCTGGAAGGT AAAACTAAGG ATGTCAGCAG 1560  
 AGAAATTTTT CCACCATTGG TGCTTGGTCA AAGAGGAAAC 1600  
 TGATGAGCTC ACTCTAGATG AGAGAGCAGT GAGGGAGAGA 1640  
 CAGAGACTCG AATTTCCGGA GCTATTTTCA TTTTCTTTTC 1680  
 CGTTTTGTGC AATTTCACTT ATGATACCGG CCAATGCTTG 1720  
 GTTGCTATTT TGGAAACTCC CCTTAGGGGA TGCCCCCTCA 1760  
 CTGGCCCTAT AAAGGGCCAG CCTGAGCTGC AGAGGATTCC 1800  
 TGCAGAGGAT CAAGACAGCA CGTGGACCTC GCACAGCCTC 1840  
 TCCCACA 1847

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 2308 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:29:

GGTACCTGCC	ACCATGGCGA	AGGGCGAGGA	ACTGTTCACT	40
GGCGTGGTCC	CAATTCTCGT	GGAAGTGGAT	GGCGATGTGA	80
ATGGGCACAA	ATTTTCTGTC	AGCGGAGAGG	GTGAAGGTGA	120
TGCCACATAC	GGAAAGCTCA	CCCTGAAATT	CATCTGCACC	160
ACTGGAAAGC	TCCCTGTGCC	ATGGCCAACA	CTGGTCACTA	200
CCTTCACCTA	TGGCGTGCAG	TGCTTTTCCA	GATACCCAGA	240
CCATATGAAG	CAGCATGACT	TTTTCAAGAG	CGCCATGCCC	280
GAGGGCTATG	TGCAGGAGAG	AACCATCTTT	TTCAAAGATG	320
ACGGGAACTA	CAAGACCCGC	GCTGAAGTCA	AGTTCGAAGG	360
TGACACCCTG	GTGAATAGAA	TCGAGTTGAA	GGGCATTGAC	400
TTTAAGGAAG	ATGGAAACAT	TCTCGGCCAC	AAGCTGGAAT	440
ACAACTATAA	CTCCCACAAT	GTGTACATCA	TGGCCGACAA	480
GCAAAAGAAT	GGCATCAAGG	TCAACTTCAA	GATCAGACAC	520
AACATTGAGG	ATGGATCCGT	GCAGCTGGCC	GACCATTATC	560
AACAGAACAC	TCCAATCGGC	GACGGCCCTG	TGCTCCTCCC	600
AGACAACCAT	TACCTGTCCA	CCCAGTCTGC	CCTGTCTAAA	640
GATCCCAACG	AAAAGAGAGA	CCACATGGTC	CTGCTGGAGT	680
TTGTGACCGC	TGCTGGGATC	ACACATGGCA	TGGACGAGCT	720
GTACAAGTGA	GCGCCTTAAG	GGCCATATGG	TGAGTGGATG	760
CCTTGACCCC	AGGCGGGGAT	GGGGGAGACC	TGTAGTCAGA	800
GCCCCCGGGC	AGCACAGGCC	AATGCCCCGTC	CTTCCCCTGC	840
AGGATGAGTA	GTGAGTGCCT	CTCCTGGCCC	TGGAAGTTGC	880
CACTCCAGTG	CCCACCAGCC	TTGTCCTAAT	AAAATTAAGT	920
TGCATCATTT	TGTCTGACTA	GGTGTCTCT	ATAATATTAT	960
AAGCTTGATA	TCGAATTCTT	TCGGACTTTT	GAAAGTGATG	1000
GTGGTGGGGG	AAGGATTCGA	ACCTTCGAAG	TCGATGACGG	1040
CAGATTTAGA	GTCTGCTCCC	TTTGGCCGCT	CGGGAACCCC	1080
ACCACGGGTA	ATGCTTTTAC	TGGCCTGCTC	CCTTATCGGG	1120
AAGCGGGGCG	CATCATATCA	AATGACGCGC	CGCTGTAAAG	1160
TGTTACGTTG	AGAAAGAATT	CCTGCAGCCC	GCCGCGTTGC	1200
TGGCGTTTTT	CCATAGGCTC	CGCCCCCCTG	ACGAGCATCA	1240
CAAAAATCGA	CGCTCAAGTC	AGAGGTGGCG	AAACCCGACA	1280
GGACTATAAA	GATACCAGGC	GTTTCCCCCT	GGAAGCTCCC	1320
TCGTGCGCTC	TCCTGTTCCG	ACCCTGCCGC	TTACCGGATA	1360
CCTGTCCGCC	TTTCTCCCTT	CGGGAAGCGT	GGCGCTTTCT	1400
CAATGCTCAC	GCTGTAGGTA	TCTCAGTTCG	GTGTAGGTCTG	1440
TTTCGCTCCAA	GCTGGGCTGT	GTGCACGAAC	CCCCCGTTCA	1480
GCCCGACCGC	TGCGCCTTAT	CCGTAACCTA	TCGTCTTGAG	1520
TCCAACCCGG	TAAGACACGA	CTTATCGCCA	CTGGCAGCAG	1560

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CCACTGGTAA	CAGGATTAGC	AGAGCGAGGT	ATGTAGGCGG	1600
TGCTACAGAG	TTCTTGAAGT	GGTGGCCTAA	CTACGGCTAC	1640
ACTAGAAGGA	CAGTATTTGG	TATCTGCGCT	CTGCTGAAGC	1680
CAGTTACCTT	CGGAAAAAGA	GTTGGTAGCT	CTTGATCCGG	1720
CAAACAAACC	ACCGCTGGTA	GCGGTGGTTT	TTTTGTTTGC	1760
AAGCAGCAGA	TTACGCGCAG	AAAAAAAGGA	TCTGGGGGAT	1800
CCGGAGAGCT	CCCAACGCGT	TGGATGCATG	GATGAGGGAA	1840
AGGAGGTAAG	ATCTGTAATG	AATAAGCAGG	AACTTTGAAG	1880
ACTCAGTGAC	TCAGTGAGTA	ATAAAGACTC	AGTGACTIONCT	1920
GATCCTGTCC	TAACTGCCAC	TCCTTGTTGT	CCCAAGAAAG	1960
CGGCTTCCTG	CTCTCTGAGG	AGGACCCCTT	CCCTGGAAGG	2000
TAAAACTAAG	GATGTCAGCA	GAGAAATTTT	TCCACCATTG	2040
GTGCTTGGTC	AAAGAGGAAA	CTGATGAGCT	CACTCTAGAT	2080
GAGAGAGCAG	TGAGGGAGAG	ACAGAGACTC	GAATTTCCGG	2120
AGCTATTTCA	GTTTTCTTTT	CCGTTTTGTG	CAATTTCACT	2160
TATGATACCG	GCCAATGCTT	GGTTGCTATT	TTGGAAACTC	2200
CCCTTAGGGG	ATGCCCCTCA	ACTGGCCCTA	TAAAGGGCCA	2240
GCCTGAGCTG	CAGAGGATTC	CTGCAGAGGA	TCAAGACAGC	2280
ACGTGGACCT	CGCACAGCCT	CTCCCACA		2308

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ART 34

1. A humanized polynucleotide vector comprising:  
a human derived promoter or mammalian homolog thereof which is functional in a target tissue or target cells, said promoter operably linked to a sequence acceptance site which directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, said vector lacking nucleic acid sequences encoding vector-derived polypeptides wherein, said vector lacks an antibiotic resistance encoding nucleic acid sequence.
2. The humanized polynucleotide vector according to claim 1 wherein the target cells are selected from the group consisting of myocytes and professional antigen presenting cells.
3. The humanized polynucleotide vector according to claim 1 or 2 wherein the target cells or target tissue are human.
4. The humanized polynucleotide vector according to claims 1-3 wherein the human derived promoter is a RANTES promoter or portion thereof.
5. The humanized polynucleotide vector according to claim 4 wherein the promoter has approximately 440 base pairs.
6. The humanized polynucleotide vector according to claims 4 or 5 wherein the portion corresponds to a region spanning the NCO site through the KpnI site of the genomic RANTES promoter
8. The humanized polynucleotide vector according to claims 1-5 or 6 further comprising an origin for replication and growth and a nucleic acid sequence which allows for selection of recombinant plasmids.
9. The humanized polynucleotide vector according to claim 8 wherein the origin for replication is colE1 or functional portion thereof.

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10. The humanized polynucleotide vector according to claim 8 wherein the origin for replication comprises a 635 base pair region of the colE1 origin of replication.

11. The humanized polynucleotide vector according to claim 1 to 6 or 8-10 further comprising a human-derived 3' splice sequence and a human-derived poly A sequence, both sequences located downstream of the sequence acceptance site.

12. The humanized polynucleotide vector according to claim 11 wherein the human derived 3' splice and poly A sequence are derived from human growth hormone.

13. A polynucleotide vector according to claims 1-6 or 8-12 wherein a 5' sequence acceptance site reads on the positive strand as GCCACCATGGCC.

14. A polynucleotide vector comprising SEQ ID No 16, SEQ ID No 27 or SEQ ID No 28.

15. A polynucleotide vector contained within a host cell deposited with the ATCC under the ATCC designation 98400 or ATCC designation 98401.

16. A polynucleotide vector according to claims 1-6 or 8-15 further comprising cDNA target products, and an optional internal ribosomal entry site, said cDNA target products integrated into said sequence acceptance site, said cDNA target products comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof alone or in combination with a nucleotide sequence encoding a cytokine or chemokine.

17. A polynucleotide vector vaccine comprising a human derived promoter or mammalian homolog thereof which is functional in a mammalian target tissue or mammalian target cell, said promoter operably linked to a sequence

acceptance site which directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, an optional internal ribosomal entry site, and cDNA target products, said cDNA target products integrated into said sequence acceptance site, said cDNA target products comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof, and said vector lacking nucleic acid sequences encoding vector-derived polypeptides wherein, said vector lacks an antibiotic resistance encoding nucleic acid sequence.

18. A polynucleotide vector vaccine according to claim 17 wherein the target antigen is a product of a tumor associated genetic derangement.

19. A polynucleotide vector vaccine according to claim 17 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen, or parasitic antigen.

20. The polynucleotide vector vaccine according to claims 17 or 18, wherein the tumor antigen is p53, RB, ras, int-2, Hst, Trc17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAP, MEN-1, ERB-B1 or combinations thereof.

21. A polynucleotide vector vaccine according to claim 17, 18, 19 or 20 further comprising an additional cDNA target product comprising a nucleic acid sequence encoding a cytokine or chemokine.

22. A polynucleotide vector vaccine according to claim 21 wherein the cytokine is selected from the group consisting of interleukin 2, interleukin 3, interleukin 4, interleukin 7, interleukin 8, interleukin 12, interleukin 15, GM-CSF, tumor necrosis factor, and interferon.

23. A polynucleotide vector vaccine according to claim 21 wherein the chemokine is selected from the group consisting of RANTES, MCP, MIP-1 $\alpha$ , MIP-1 $\beta$ , defensins, IP-10 and combinations thereof.

24. A method for expressing at least one target antigen or antigenic epitope thereof in cells comprising:

introducing a humanized polynucleotide vector into said cells, under conditions for expression of the target antigen or antigenic epitope thereof, said vector comprising:

a human derived promoter or mammalian homolog thereof, which is functional in said cells, said promoter operably linked to a sequence acceptance site which directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease and,

cDNA target products, and an optional internal ribosomal entry site, said cDNA target products integrated into said sequence acceptance site, said cDNA target products comprising a nucleic acid sequence encoding at least one target antigen or antigenic epitope thereof, and said vector lacking nucleic acid sequences encoding vector-derived polypeptides, wherein said vector lacks an antibiotic resistance encoding nucleic acid sequence.

25. The method of claim 24 wherein the cells are selected from the group consisting of myocytes and professional antigen presenting cells.

26. The method of claim 24 wherein the target antigen is a tumor antigen bacterial antigen, viral antigen, or parasitic antigen.

27. The method of claim 26 wherein the tumor antigen is p53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FLS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 or combinations thereof.

28. A pharmaceutical composition comprising at least one polynucleotide vector according to claims 1-6 or 8-16 and a pharmaceutically acceptable carrier.

29. A pharmaceutical composition comprising the polynucleotide vector vaccine according to claims 17-22 or 23 and a pharmaceutically acceptable carrier.

30. A kit comprising the polynucleotide vector according to claims 1-6 or 8-16.

32. A kit comprising the polynucleotide vector vaccine according to claims 17-22 or 23.

33. A kit according to claim 32, further comprising an expression enhancing agent.

34. The kit according to claim 33 wherein the expression enhancing agent

is a mycotoxic agent.

35. The kit according to claim 34 wherein the mycotoxic agent is bupivacaine-HCl and dextrose.

36. A host cell comprising:

the polynucleotide vector of claim 17-22 or 23, wherein the host cell is capable of expressing the target antigen or antigenic epitope.

37. The host cell according to claim 36 wherein the host cell is a myocyte or professional antigen presenting cell.

38. A method of stimulating a specific immune response to at least one target antigen or antigenic epitope thereof in a mammal comprising: administration of an effective amount of a polynucleotide vector vaccine according to claim 17-22 or 23 into the mammal, said amount elicits the specific immune response to the target antigen or epitope thereof.

39. The method according to claim 38, wherein a site of administration is muscle or skin.

40. The method according to claim 38 further comprising administration of effective amount of an expression enhancing agent prior to administration of the polynucleotide vector vaccine.

41. The method according to claim 40 wherein the expression enhancing agent is a mycotoxic agent.

42. The method according to claim 41 wherein the mycotoxic agent is bupivacaine-HCl or dextrose.

43. The method according to claim 38-41 or 42 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen or parasitic antigen.

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44. The method according to claim 43 wherein the tumor antigen is selected from the group consisting of P53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 and combinations thereof.

45. The method according to claim 44 wherein the method generates antigen specific cytotoxic lymphocytes to the tumor antigen or antigenic epitopes thereof.

46. A method of making a humanized polynucleotide vector comprising:  
operably linking a human derived promoter or mammalian homolog thereof which is functional in a target tissue or target cells to a sequence acceptance site, said site directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, said vector lacking nucleic acid sequences encoding vector-derived polypeptides wherein, said vector lacks an antibiotic resistance encoding nucleic acid sequence.

47. The method according to claim 46, wherein the human derived promoter is a RANTES promoter or portion thereof.

48. A isolate antibody comprising an antibody elicited in response to immunization with the polynucleotide vector vaccine according to claim 17-22 or 23, said antibody is specific for the target antigen or antigenic epitope thereof expressed by the mammalian target tissue or mammalian target cell.

50. The sequence acceptance site comprising nucleic acid sequences which accept cDNA target products from rtPCR cloning wherein the sequence acceptance site directionally accepts target sequence specific products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease.

51. The sequence acceptance site according to claim 50 wherein the restriction endonuclease is Bgl I.

52. The sequence acceptance site according to claim 50 or 51 wherein

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a 5' acceptance site reads on the positive strand as GCCACCATGGCC.

53. The sequence acceptance site according to claim 52 wherein a 3' acceptance site reads on the positive strand as GCCTTAAGGGC.

54. The sequence acceptance site according to claim 50 wherein the site comprises the nucleotide sequence as depicted in Figure 2.

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Figure 1. Schematic representation of the experimental design. The subjects were divided into two groups: the control group (CG) and the experimental group (EG). The CG was divided into two subgroups: the control group (CG) and the control group (CG). The EG was divided into two subgroups: the experimental group (EG) and the experimental group (EG). The subjects were divided into two groups: the control group (CG) and the experimental group (EG). The CG was divided into two subgroups: the control group (CG) and the control group (CG). The EG was divided into two subgroups: the experimental group (EG) and the experimental group (EG).

65. The humanized polynucleotide vector according to claim 8, wherein the

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nucleic acid sequence which allows for selection is a suppressor tRNA gene, a synthetic SupF complementation tRNA gene, or functional derivatives thereof.

66. The humanized polynucleotide vector according to claim 65, wherein the nucleic acid sequence is selected from the group consisting of SupE, SupP, SupD, SupU, SupF, SupZ, glyT, glyU, SerP,  $psu^{+}$ ,  $psu^{+}$ -C34,  $psu^{+}$ AM and  $psu^{+}$ OC.

67. A polynucleotide vector according to claims 1-6 or 8-12 wherein a 3' sequence acceptance site reads on the positive strand as GCCTTAAGGGC.

68. The humanized polynucleotide vector according to claims 1-6 or 8-13 wherein the sequence acceptance site comprises the nucleotide sequence as depicted in Figure 2.

69. The method according to any of claims 24 through 27 wherein the method is ex vivo.

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STATEMENT UNDER ARTICLE 19

In response to the Notification of Transmittal of the International Search Report or Declaration dated November 27, 1997, Applicants are forwarding herein replacement sheets of amended claims.

Claims 1, 17, 24 and 46 were amended to describe the sequence acceptance site as recited in claim 50. Claims 1, 17, 24 and 46 were further amended to recite "lacking nucleic acid sequences encoding vector-derived polypeptides."

Claims 20 and 22 were amended to correct grammatical errors.

Claim 23 was amended to correct a typographical error in MIP-1 $\alpha$  and to insert the chemokine "IP-10".

The recitation from cancelled claim 49 was incorporated into claim 50.

Claim 52 was amended to reflect the cancellation of claim 49.

Claim 54 was amended to depend from claim 50.

New "use of product" claims were added, i.e. claims 55-62 which mirror the method of treatment claims, claims 38-45.

New claims 63-66 were added which further characterize the vector.

Applicants respectfully submit that the amendment does not introduce new matter and are fully supported by, and do not change the meaning or content of the description and drawings.